FORM PTO-1390U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 5-93) **TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371**	ATTORNEY'S DOCKET NUMBER 9013-36
	U S APPLICATION NO (If known, see 37 C.F.R. 1 5) **09/936029**

INTERNATIONAL APPLICATION NO. PCT/GB00/00860	INTERNATIONAL FILING DATE March 9, 2000	PRIORITY DATE CLAIMED March 3, 1999

TITLE OF INVENTION

NEURODEGENERATIVE DISORDER RELATED GENE

APPLICANT(S) FOR DO/EO/US

Roger W. Davies, Anthony P. Payne, Roger G. Sutcliffe

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
    a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
    b. [ ] has been transmitted by the International Bureau.
    c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).

6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
    a. [X] are transmitted herewith (required only if not transmitted by the International Bureau).
    b. [ ] have been transmitted by the International Bureau.
    c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
    d. [ ] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [X] Other items or information: International Preliminary Examination Report

U.S. APPLICATION NO (If known, see 37 C F R. 1 50)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/936029		9013-36

17. [ ] The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $840.00	840.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482). $670.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $690.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO. $970.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4). $96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	44-20 =	22	X $18.00	$396.00	
Independent Claims	11-3 =	8	X $78.00	$624.00	
Multiple dependent claim(s) (if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$1860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$1860.00	
				Amount to be refunded	$
				charged	$

a. [X] A check in the amount of **$1860.00** to cover the above fees is enclosed.
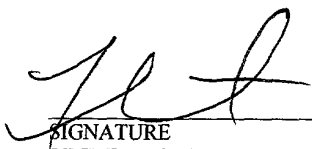
b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0220.
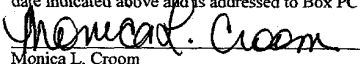
NOTE: **Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO: F. Michael Sajovec

20792
PATENT TRADEMARK OFFICE

SIGNATURE
REGISTRATION NO.: 31,793

CERTIFICATE OF EXPRESS MAILING
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    I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Commissioner for Patents, Washington, DC 20231.

Monica L. Croom   Date of Signature: September 7, 2001

PTO FORM-1390 (REV 5-93)

Attorney's Docket No. 9013-36   PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Davies et al.
Serial No.: to be assigned
Filed: concurrently herewith
For: *NEURODEGENERATIVE DISORDER RELATED GENE*

Date: September 7, 2001

Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Please enter the following Preliminary Amendment before examining the present application. Attached hereto at page five is a marked-up version of the changes made to the claims by the current amendment. The marked-up version of the changes is captioned **"Version With Markings To Show Changes Made."**

In the specification:

Please amend the specification as follows:

At page one, below the title "NEURODEGENERATIVE DISORDER RELATED GENE," please insert -- This application claims priority from Great Britain Application No. 9905218.5 and PCT Application No. PCT/GB00/00860, filed March 9, 2000, both in English, the disclosure of which are incorporated by reference herein in their entirety.--

In the Claims:

Please amend Claims 4, 8, 12, 16-17, 20, 22, 26-27, 30-31, 41, and 43-44 as follows.

4. (Amended) Use of a polynucleotide fragment according to claim 1 wherein the degenerative disorder is a degenerative disorder of the central nervous system.

8. (Amended) Use of a polynuceotide fragment according to claim 6 wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy,

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Progressive supranuclear palsy, cortical-basal ganglionic (corticobasal) degeneration, vascular Parkinsonism and ballism.

12. (Amended) Use of a polypeptide according to claim 9 wherein a degenerative disorder is a degenerative disorder of the central nervous system.

16. (Amended) Use of a polypeptide according to claim 15 wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear palsy, cortical-basal ganglionic (corticobasal) degeneration, vascular Parkinsonism and ballism.

17. (Amended) Use of a polypeptide according to claim 9 wherein the polypeptide is synthetic.

20. (Amended) A method according to claim 18 wherein the animal is a mammal.

22. (Amended) A method according to claim 18 wherein the neurodegenerative disorder is a degenerative disorder of the central nervous system.

26. (Amended) A method according to claim 24 wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear palsy, cortical-basal ganglionic (corticobasal) degeneration, vascular Parkinsonism and ballism.

27. (Amended) A method according to claim 18 wherein the mutation results in a truncated product from the PKCγ gene being produced.

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30. (Amended) A method according to claim 18 wherein detection of the presence of the mutation in the PKCγ gene is achieved by detecting altered levels of the mRNA transcripts or mRNA precursor.

31. (Amended) A method according to claim 18 wherein the mutation in the PKCγ gene is detected using antibodies raised to the truncated PKC type I polypeptide.

41. (Amended) An antibody according to claim 38 wherein the antibody is a monoclonal antibody.

43. (Amended) Use of an antibody according to claim 38 for the manufacture of a medicament for preventing, delaying, treating or inhibiting degeneration of the nervous system.

44. (Amended) Use of an antibody according to claim 38 in a diagnostic assay for testing an human thought to have or be predisposed to having a neural degenerative disorder.

Abstract:

At page 58, the page following the claims, please insert —The present invention relates to the use of a polynucleotide fragment comprising PKCγ gene including type 1 subtype of protein kinase C in the manufacture of a medicament for treating a neurodegenerative disorder. The invention further relates to use of a polypeptide which comprises protein kinase C type 1 in the manufacture of a medicament for treating a neurodegenrative disorder. Further disclosed is a method of testing an animal, such as human, thought to have or be predisposed to having a neurodegenerative disorder which comprises detecting the presence of a mutation in PKCγ gene and/or its associated promoter.

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Remarks

The above amendments are made to more clearly define the invention under United States practice. Please enter this amendment prior to calculation of the filing fee.

Respectfully submitted,

F. Michael Sajovec
Registration No. 31,793

USPTO Customer No.:

20792
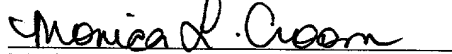
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    I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: BOX PATENT APPLICATION, Commissioner for Patents, Washington, DC 20231.

Monica L. Croom
September 7, 2001

In re: Davies et al.
Serial No.: to be assigned
Filed: concurrently herewith
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Version With Markings to Show Changes

In the specification:

Please amend the specification as follows:

At page one, below the title "NEURODEGENERATIVE DISORDER RELATED GENE," please insert -- <u>This application claims priority from PCT Application No. PCT/GB00/00860, filed March 9, 2000, the disclosure of which is incorporated by reference herein in its entirety.</u>--

In the Claims:

Please amend Claims 4, 8, 12, 16-17, 20, 22, 26-27, 30-31, 41, and 43-44.

4. (Amended) Use of a polynucleotide fragment according to [any preceding claim] <u>claim 1</u> wherein the degenerative disorder is a degenerative disorder of the central nervous system.

8. (Amended) Use of a polynuceotide fragment according to [either of claims 6 or 7] <u>claim 6</u> wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear palsy, cortical-basal ganglionic (corticobasal) degeneration, vascular Parkinsonism and ballism.

12. (Amended) Use of a polypeptide according to [any of claims 9 to 11] <u>claim 9</u> wherein a degenerative disorder is a degenerative disorder of the central nervous system.

16. (Amended) Use of a polypeptide according to [either of claims 14 or 15] <u>claim 15</u> wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear palsy, cortical-basal ganglionic (corticobasal) degeneration,

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vascular Parkinsonism and ballism.

17. (Amended) Use of a polypeptide according to **[any of claims 9 to 16]** <u>claim 9</u> wherein the polypeptide is synthetic.

20. (Amended) A method according to **[either of claims 18 or 19]** <u>claim 18</u> wherein the animal is a mammal.

22. (Amended) A method according to **[either of claims 18 and 19]** <u>claim 18</u> wherein the neurodegenerative disorder is a degenerative disorder of the central nervous system.

26. (Amended) A method according to **[either of claims 24 or 25]** <u>claim 24</u> wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear palsy, cortical-basal ganglionic (corticobasal) degeneration, vascular Parkinsonism and ballism.

27. (Amended) A method according to **[either of claims 18 or 19]** <u>claim 18</u> wherein the mutation results in a truncated product from the PKCγ gene being produced.

30. (Amended) A method according to **[either of claims 18 or 19]** <u>claim 18</u> wherein detection of the presence of the mutation in the PKCγ gene is achieved by detecting altered levels of the mRNA transcripts or mRNA precursor.

31. (Amended) A method according to **[either of claims 18 or 19]** <u>claim 18</u> wherein the mutation in the PKCγ gene is detected using antibodies raised to the truncated PKC type I polypeptide.

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41. (Amended) An antibody according to [any of claims 38 to 40] claim 38 wherein the antibody is a monoclonal antibody.

43. (Amended) Use of an antibody according to [claims 38-42] claim 38 for the manufacture of a medicament for preventing, delaying, treating or inhibiting degeneration of the nervous system.

44. (Amended) Use of an antibody according to [claims 38-47] claim 38 in a diagnostic assay for testing an human thought to have or be predisposed to having a neural degenerative disorder.

Abstract:

At page 58, the page following the claims, please insert --The present invention relates to the use of a polynucleotide fragment comprising PKCγ gene including type 1 subtype of protein kinase C in the manufacture of a medicament for treating a neurodegenerative disorder. The invention further relates to use of a polypeptide which comprises protein kinase C type 1 in the manufacture of a medicament for treating a neurodegenrative disorder. Further disclosed is a method of testing an animal, such as human, thought to have or be predisposed to having a neurodegenerative disorder which comprises detecting the presence of a mutation in PKCγ gene and/or its associated promoter.--

NEURODEGENERATIVE DISORDER RELATED GENE

The present invention relates to the use of a
polynucleotide fragment encoding protein kinase C type I
(PKC type I) as well as fragments thereof, mutant
polynucleotide fragments of the polynucleotide fragment, a
recombinant vector comprising such a polynucleotide
fragment or mutant polynucleotide fragment, a host cell
comprising said polynucleotide fragment or mutant
polynucleotide fragment, a host cell comprising a
recombinant vector comprising said polynucleotide fragment
or mutant polynucleotide fragment, a recombinant or
synthetic polypeptide thereto, antibodies specific to said
polypeptide, antisense oligonucleotides complementary to
said polynucleotide fragment or mutant polynucleotide
fragment, pharmaceutical compositions comprising said
recombinant or synthetic polypeptide, pharmaceutical
compositions comprising said antisense oligonucleotides and
pharmaceutical compositions comprising said polynucleotide
fragment for use in prophylaxis and/or as a therapeutic
agent in animals, particularly humans, as well as uses of
said polynucleotide fragment or mutant polynucleotide
fragment, antisense oligonucleotides, antibodies and/or
polypeptides in diagnostic and/or screening assays.

Degenerative disorders of the nervous system, such as
Parkinson's Disease, Alzheimer's Disease and Huntington's
Disease, have provided a challenge for many years, in both
the basic research and clinical contexts. A major problem
has been the lack of animal models which accurately mimic

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the clinical conditions since a large proportion of research is carried out initially on animals in which a disorder has been created by experimental manipulation of the central nervous system (CNS). Examples of experimental manipulation of the CNS in the field of Parkinson's Disease

5   include rodent studies which have relied on lesioning the nigrostriatal system with, for example, the toxin 6-hydroxydopamine. Several genetic mouse models of movement disorders exist, although the majority of such mutants breed poorly and have a reduced life expectancy which

10   limits their efficacy for study of the long term progression of the various conditions. One such model, the weaver mouse, has a deficiency in its dopaminergic systems and has thus been proposed as a model of Parkinson's disease. However, this mutant also possesses severe

15   cerebellar abnormalities and the resulting behaviours may mask those generated by the Parkinsonian-like dopamine deficiencies.

A mutant rat strain (AS/AGU) which had risen spontaneously in a closed breeding colony of Albino-Swiss

20   (AS) rats at the Department of Anatomy, Glasgow University (AGU) was initially described by Clarke & Payne (1994) European Journal of Neuroscience 6 pp885 - 888. The mutant rat displayed a movement disorder which consisted primarily of a difficulty in initiating movement, with a staggering

25   gait and hind limb rigidity. The animals were in good general health and were fertile. Successful breeding between affected individuals resulted after several

generations in all off-spring bearing the same motor deficits. Subsequent genetic analysis has shown that the mutation is an autosomal recessive. The gait disturbances are first detected at around postnatal day 10 and become progressively more severe. The life expectancy of these animals is around 18 months, somewhat foreshortened when compared with the parent Albino-Swiss strain, whose life expectancy is more than 2 years.

A 60% deficit in dopaminergic cell bodies in the substantia nigra pars compacta was detected in the AS/AGU mutants compared to the AS controls at 12 months of age. This provided evidence for basal ganglia involvement and suggested that the disorder could be pathologically very similar to human Parkinson's Disease, which is characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc).

Further research using the micropunch procedure revealed depletions of tissue (combined pre-synaptic and released) dopamine in the dorsal and lateral striatum of 30% and 20% respectively in 12 month old AS/AGU mutants compared to age matched controls (Campbell et al 1996 Neuroscience Letters, 213 pp 173 - 176). This was an expected consequence of loss or decreased function of dopaminergic neurons in the SNpc, which project to the striatum.

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When an age range study was carried out on rats of 3 months, 6 months, 9 months and 12 months old, it was found that tissue dopamine depletion in the dorsal and lateral striatum of AS/AGU mutants increased with age from 6 months onwards, thereby demonstrating that the disorder was progressive (Campbell et al, 1997 Neuroscience Letters 239 pp54 - 56).

Extracellular levels of dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) were measured by microdialysis in the corpus striatum of conscious AS/AGU mutant rats. Extracellular levels of dopamine were found to be very significantly reduced-approximately 80% in 9 month old AS/AGU rats compared to age matched AS controls. This was also found to be progressive over an age range. The extracellular levels of the degradation product of dopamine, ie. DOPAC, was found to be elevated in AS/AGU rats compared to AS controls at all ages (Campbell et al, 1998 Neuroscience 85 pp323 - 325).

Local cerebral glucose utilisation is a measure of the metabolic activity of cells in various brain regions. This was measured in AS/AGU rats and statistically significant decreases in glucose utilisation were apparent in 12 out of 44 brain regions examined in 12 month rats. The most significant decreases were found in the substantia nigra pars compacta and the medidical geniculate. Lesser effects were observed in the subthalamic nucleus in extra pyramidal regions and several limbic structures. The cerebellum and white matter areas were not affected. This evidence

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suggests that the dopaminergic cells of the SNpc are in some way metabolically comprised (Lam et al, 1998 European Journal of Neuroscience 10 pp1963 - 1967).

When L-Dopa was administered to AS/AGU rats it was shown to greatly enhance the ability of the AS/AGU rats to perform a number of locomotor tasks such as mid-air righting and walking down an inclined ramp. This was also observed when foetal midbrain cells were transplanted into the striatum. L-Dopa treatment and foetal midbrain transplants are known to improve the symptomatic state of human Parkinson's Disease patients. This result revealed that the majority of the movement disorder, and thus the neurodegenerative damage in the AS/AGU is due to loss of dopaminergic neuron function in the SNpc (Payne et al, 1998 Movement Disorders 13 pp832 - 834).

All this work suggested that the AS/AGU rat may be a good candidate as a phenotypic model for Parkinson's Disease. However, there was no evidence of how the AS/AGU rat may be affected at the genetic level.

The PKC gamma gene encoding the protein kinase C type I (PKC type I) isoenzyme has previously been studied in mice and transgenic mice lacking the type I subtype have been produced (Abeliovich et al 1993, Cell, 75, pp1253 - 1262). The null mutant mice produced displayed little or no behavioural impairment.

A mutation in the PKCγ gene in humans has been shown to be associated with the disorder retinitis pigmentosa (RP) see Al-Maghtheh et al, 1998 Am. J. Hum. Genetics 62

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pp1248 - 1252. However, there was no suggestion that the mutation was associated with any additional neurological disorder such as Parkinson's Disease, Alzheimer's Disease or Huntington's Disease.

The present invention is based on the discovery by the present inventors that a mutation(s) within the PKCγ gene encoding the type I subtype of protein kinase C is associated with the AS/AGU mutant rat.

Thus, in a first aspect, the present invention provides use of a polynucleotide fragment comprising the PKCγ gene encoding the type I subtype of protein kinase C in the manufacture of a medicament for treating a neurodegenerative disorder.

In a further aspect, the present invention provides use of a polypeptide which comprises protein kinase C type I in the manufacture of a medicament for treating a neurodegenerative disorder.

Typically the medicament may be used to treat mammals, in particular humans. The neurodegenerative disorder may be a degenerative disorder of the central nervous system, such as Alzheimer's Disease, or more particularly, a neurodegenerative disorder associated with dopaminergic cell degeneration and/or movement impairments such as Parkinson's Disease, or Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy (including striatonigral degeneration, sporadic olivopontocerebellar atrophy and shy-drager syndrome), Progressive supranuclear palsy, cortical-basal ganglionic

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(corticobasal) degeneration, vascular Parkinsonism or ballism.

"Polynucleotide fragment" as used herein refers to a polymeric form of nucleotides of any length, both to ribonucleic acid sequences and to deoxyribonucleic acid sequences. In principle, this term refers to the primary structure of the molecule, thus this term includes double stranded and single stranded DNA, as well as double and single stranded RNA, and modifications thereof.

In general, the term "polypeptide" refers to a molecular chain of amino acids with a biological activity. It does not refer to a specific length of the product, and if required it can be modified *in vivo* and/or *in vitro*, for example by glycosylation, myristoylation, amidation, carboxylation or phosphorylation; thus *inter alia* peptides, oligopeptides and proteins are included. The polypeptides disclosed herein may be obtained, for example, by synthetic or recombinant techniques known in the art.

Thus the term extends to cover, for example, polypeptides obtainable from various transcripts and splice variants of these transcripts from the PKCγ gene. Additionally, functional domains may be observed in the protein and isolated polypeptides relating to these functional domains may be of particular use. For example, a regulatory domain, a kinase domain and an ATP-binding domain have been observed in the PKC type I polypeptide. The present invention also relates to polynucleotide fragments comprising a nucleotide sequence encoding such

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functional domain polypeptides.

It will be understood that for the PKCγ nucleotide and polypeptide sequences referred to herein, natural variations can exist between individuals. These variations may be demonstrated by amino acid differences in the overall sequence or by deletions, substitutions, insertions or inversions of amino acids in said sequence. All such variations are included in the scope of the present invention.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in a different codon encoding the same amino acid. Consequently, it is clear that any such derivative nucleotide sequence based on the sequences disclosed herein is also included in the scope of the present invention.

Thus, the present invention also includes nucleotide sequences similar to the polynucleotide sequences disclosed herein. It is understood that similar sequences include sequences which remain hybridised to the polynucleotide sequences of the present invention under stringent conditions. Typically, a similar test sequence and a polynucleotide sequence of the present invention are allowed to hybridise for a specified period of time generally at a temperature of between 50 and 70°C in double strength SSC (2 x NaCl 17.5g/1 and sodium citrate (SC) at 8.8 g/1) buffered saline containing 0.1% sodium dodecyl sulphate (SDS) followed by rinsing of the support at the same temperature but with a buffer having a reduced SSC

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concentration. Depending upon the degree of similarity of the sequences, such reduced concentration buffers are typically single strength SSC containing 0.1% SDS, half strength SSC containing 0.1% SDS and one tenth strength SSC containing 0.1% SDS. Sequences having the greatest degree of similarity are those the hybridisation of which is least affected by washing in buffers of reduced concentration. It is most preferred that the similar and inventive sequences are so similar that the hybridisation between them is substantially unaffected by washing or incubation in one tenth strength SSC containing 0.1% SDS.

Furthermore, fragments derived from the PKCγ gene or PKC type I protein which still display PKCγ specific properties or PKC type I specific properties are also included in the present invention. "PKCγ specific properties" is understood to relate to biological functions which are attributable to naturally-occurring PKCγ gene and "PKC type I specific properties" is understood to relate to biological functions which are attributable to naturally-occurring PKC type I protein. This may include fusion proteins.

All such modifications mentioned above resulting in such derivatives of PKCγ are covered by the present invention so long as the characteristic PKCγ properties remain substantially unaffected in essence.

The present inventors applied genetic mapping techniques in order to ascertain the genotypic variation displayed in the AS/AGU rat using the process of "positional

cloning" (Collins, 1992, Nature Genetics, 1, 3 - 6). This
mapping revealed that the AS/AGU mutation was in close
proximity to the genetic marker R158 (Serikawa et al, 1992,
Genetics 137, pp701 - 721). Nucleotide sequencing was then
carried out, which upon comparison with wild type AS

5   sequence, revealed a mutation in the PKCγ gene.

A point mutation was observed at nucleotide 841 of the
rat PKCγ messenger RNA sequence(shown in Figure 1 where
nucleotide numbering is such that base A of the ATG start
codon is no. 1) such that a guanine base, present in the AS

10   gene sequence, was mutated to a thymine base in the AS/AGU
mutant sequence. This transversion mutation results in the
generation of an in-frame stop codon which upon translation
of the PKCγ gene would result in a prematurely terminated
protein the length of which would be 280 amino acids. It

15   is postulated that this truncated protein would not possess
several of the domains present in the wild-type protein.
That is, the regulatory domain would be present in the
truncated protein and not the kinase or ATP-binding
domains.

20   Since this genotypic mutation is associated with the
phenotypic movement disorder observed in the AS/AGU mutant
rat, observation of such a mutation in PKCγ may be used in
a predictive test for neurodegenerative disorders, such as
Parkinson's disease, Alzheimer's Disease or Huntington's

25   Disease. Alternatively, measuring levels of PKC type I
protein levels and/or activity may be useful in a
predictive or diagnostic test for neurodegenerative

disorders.

Thus, in a further aspect, the present invention provides a method of testing an animal thought to have or be predisposed to having a neurodegenerative disorder which comprises detecting the presence of a mutation in the PKCγ gene and/or its associated promoter.

Typically, the mutation(s) may result in a truncated product from the PKCγ gene being produced. More particularly the mutation may occur in the 5' half of the gene. For example the mutation may be a point mutation such as at position 841 of the rat PKCγ gene or similar region of the PKCγ gene from another species. The skilled man will immediately appreciate that the information presented herein relating to the rat PKCγ may easily be equated or correlated with a similar mutation at a corresponding location in the PKCγ gene from another species, such as humans. Thus, the present invention provides the means with which to test humans for a similar mutation in the human PKCγ gene and therefore predict if the test subject has or is predisposed to developing a neurodegenerative disorder for example, Parkinson's Disease, Alzheimer's Disease or Huntington's Disease.

Typical techniques for detecting the mutation may include restriction fragment length polymorphism, hybridisation techniques, DNA sequencing, exonuclease resistance, microsequencing, solid phase extension using ddNTPs, extension in solution using ddNTPs, oligonucleotide ligation assays, methods for detecting single nucleotide

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polymorphisms such as dynamic allele-specific hybridisation, ligation chain reaction, mini-sequencing, DNA "chips", allele-specific oligonucleotide hybridisation with single or dual-labelled probes merged with PCR or with molecular beacons, and others.

5   Altered levels of the mRNA transcripts encoding the truncated PKC type I polypeptide have been observed. Thus, detection of altered levels of the mRNA transcript or mRNA precursors, such as nascent RNA, may be used to diagnose if the test subject has or is predisposed to developing a

10   neurodegenerative disorder.

The information presented herein may also be used to genetically manipulate the wild-type PKCγ gene, mutant PKCγ gene or derivatives thereof, for example to clone the gene by recombinant DNA techniques generally known in the art.

15   Cloning of homologous genes from other species of mammal may be performed with this information by widely known techniques; for example, suitable primers may be designed to a consensus region and/or functional domains of the sequence shown in Figure 2 and such primers used as probes

20   for cloning homologous genes from other organisms.

Moreover, mammalian PKCγ mutant and wild-type nucleotide sequences of the present invention are preferably linked to expression control sequences. Such control sequences may comprise promoters, operators,

25   inducers, ribosome binding sites etc. Suitable control sequences for a given host may be selected by those of ordinary skill in the art.

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A nucleotide sequence according to the present invention can be ligated to various expression-controlling DNA sequences, resulting in a so-called recombinant nucleic acid molecule. Thus, the present invention also includes an expression vector comprising an expressible PKCγ mutant or wild-type nucleotide sequence. Said recombinant nucleic acid molecule can then be used for transformation of a suitable host.

Such recombinant nucleic acid molecules are preferably derived from for example, plasmids, or from nucleic acid sequences present in bacteriophages or viruses and are termed vector molecules.

Specific vectors which can be used to clone nucleotide sequences according to the invention are known in the art (eg. Rodriguez and Denhardt, editors, Vectors: A survey of molecular cloning vectors and their uses, Butterworths, 1988).

The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, 1989.

The present invention also relates to a transformed cell comprising the mutant or wild-type nucleic acid molecule in an expressible form. "Transformation", as used herein, refers to the introduction of a heterologous nucleic acid sequence into a host cell in vivo, ex vivo or

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in vitro irrespective of the method used, for example, by calcium phosphate co-precipitation, direct uptake or transduction.

The heterologous nucleic acid sequence may be maintained through autonomous replication or alternatively may be integrated into the host's genome. The recombinant DNA molecules are preferably provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted nucleic acid sequence.

The most widely used hosts for expression of recombinant nucleic acid molecules are bacteria, yeast, insect cells and mammalian cells. Each system has advantages and disadvantages in terms of the vector used, potential ease of production and purification of a recombinant polypeptide and authenticity of product in terms of tertiary structure, glycosylation state, biological activity and stability and will be a matter of choice for the skilled addressee.

In addition to promoting expression of a PKC type I polypeptide in cells, in certain circumstances it may be advantageous to substantially prevent or reduce the expression or activity of the native PKC type I in a host, for example, for the production of animal models for use in drug screening, or particularly if the native PKC type I is of a mutant form.

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Thus, according to a further aspect of the invention, there is provided an antisense nucleotide fragment complementary to a PKCγ nucleotide sequence of the present invention. Included in the scope of "antisense nucleotide fragment" is the use of synthetic oligonucleotide sequences, or of equivalent chemical entities known to those skilled in the art, for example, peptide nucleic acids. Further, such sequences can be used as part of ribozyme and/or triple helix sequences, which may also be useful for target gene regulation. Also provided is a nucleotide fragment comprising a nucleotide sequence which, when transcribed by the cell, produces such an antisense fragment. Typically, antisense RNA fragments will be provided which bind to complementary PKCγ mRNA fragments to form RNA double helices, allowing RNAse H to cleave the molecule and rendering it incapable of being translated by the cell into polypeptides.

A further aspect of the present invention provides antibodies specific to the PKC type I polypeptide or truncated polypeptide as identified herein or epitopes thereof. Production and purification of antibodies specific to an antigen is a matter of ordinary skill, and the methods to be used are clear to those skilled in the art. The term antibodies can include, but is not limited to, polyclonal antibodies, monoclonal antibodies (mAbs), humanised or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')$_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id)

antibodies, and epitope binding fragments of any of the above. Such antibodies may be used in modulating the expression or activity of the full length or truncated PKC type I polypeptide, or in detecting said polypeptide *in vivo* or *in vitro*.

5   The present invention further provides a recombinant or synthetic PKC type I polypeptide for the manufacture of reagents for use as prophylactic or therapeutic agents in mammals. In particular, the invention provides pharmaceutical compositions comprising the recombinant or

10   synthetic PKC type I polypeptide together with a pharmaceutically acceptable carrier therefor.

According to a still further aspect of the present invention, there is provided use of a polypeptide or nucleic acid sequence as hereinbefore described for

15   promoting nervous system degeneration for use in, for example, production of animal models which may be used in drug screening.

There is also provided use of a polypeptide or nucleic acid sequence as hereinbefore described in preventing,

20   delaying, treating or inhibiting degeneration of the nervous system. There is further provided a method of preventing, delaying, treating or inhibiting degeneration of the nervous system comprising providing PKC type I polypeptide to a subject displaying or predicted to display

25   degeneration of the nervous system. Such a method may find particular application in the treatment of degenerative disorders of the central nervous system, such as

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Alzheimer's Disease, or more particularly neurodegenerative disorders associated with movement impairment such as Parkinson's Disease or Huntington's Chorea.

Also provided is a method of preventing, delaying, treating or inhibiting degeneration of the nervous system comprising providing a subject with a nucleotide sequence or an antibody which substantially prevents or reduces expression or activity of a mutant PKC type I polypeptide.

A yet further aspect of the present invention provides use of polypeptides or nucleic acid sequences as hereinbefore described in the treatment of degenerative disorders of the nervous system, such as Parkinson's Disease, Alzheimer's Disease or Huntington's Disease. One such envisaged treatment may be by way of so-called gene therapy in which a wild-type PKCγ gene is introduced to a subject possessing a mutant PKCγ gene in order to counter the effects of the mutant PKCγ gene. This may be performed by the implantation of cells, such as fibroblasts expressing human or mammalian PKCγ fused to herpes virus VP22 protein that will transfer itself and PKCγ into adjacent neurons. Transformation of the cells to be implanted may be performed *in vitro* by any number of techniques, including physical means such as microinjection, electroporation, bioballistic or particle bombardment, jet injection or others; by chemical means such as using calcium phosphate, DEAE dextran, polylysine conjugates, "starburst" dendrimer conjugates, polybrene-dimethyl sulphoxide. The PKCγ gene itself, within an

appropriate vector end-linked to an appropriate expression system, may be directly delivered via receptor-mediated uptake systems such as asialoglycoprotein and transferrin, liposomes, virus-like particles, intracellular targeting ligands and others; and by biological means including

5   retroviral vectors such as Moloney murine leukaemia virus, adenovirus vectors and adeno-associated virus vectors, Herpes Simplex virus vectors, Semliki Forest virus vectors, Sindbis virus vectors and others.

The present invention also relates to methods for

10   prognostic and diagnostic evaluation of various degenerative disorders of the nervous system, and for the identification of subjects who are predisposed to such disorders, for example determination of allelic variation by determination of the PKCγ nucleotide sequence in an

15   individual and/or detection of truncated transcripts derived from the PKCγ gene, whether they are mRNA or polypeptide or measurements of PKC type I levels and/or activity. Furthermore, the invention provides methods for evaluating the efficacy of drugs for such disorders and

20   monitoring the progress of patients involved in clinical trials for the treatment of such disorders.

The invention further provides methods for the identification of compounds which modulate the expression of a mutated or wild-type PKCγ gene and/or the activity of

25   the product(s) of such a mutant or wild-type PKCγ gene which may be involved in processes relevant to degenerative disorders of the nervous system. Such compounds may

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include agonists, defined as compounds which increase the expression of a mutated or wild-type PKCγ gene and/or activity of the product(s) of such a mutant or wild-type PKCγ gene, and/or antagonists, defined as compounds which decrease the expression of a mutated or wild-type PKCγ gene

5   and/or the activity of the product(s) of such a mutant or wild-type PKCγ gene.   Thus, the present invention in a further aspect also provides agonists and/or antagonists.

The biological function of the PKCγ gene can be more directly assessed by utilizing relevant *in vivo* and *in*

10   *vitro* systems.   *In vivo* systems can include, but are not limited to, animal systems which naturally exhibit the symptoms of nervous system disorders, or ones which have been engineered to exhibit such symptoms.   Further, such systems can include, but are not limited to, transgenic

15   animal systems.   *In vitro* systems can include, but are not limited to, cell-based systems comprising PKCγ gene/PKC type I protein expressing cell types.   The cells can be wild type cells, or can be non-wild type cells containing modifications known or suspected of contributing to the

20   disorder of interest.

In further characterising the biological function of the PKCγ mutant or wild-type gene, the expression of the PKCγ mutant or wild-type gene can be modulated within the *in vivo* and/or *in vitro* systems, i.e. either overexpressed

25   or underexpressed in, for example, transgenic animals and/or cell lines, and its subsequent effect on the system can then be assayed.   Alternatively, the activity of the

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product of the identified gene can be modulated by either increasing or decreasing the level of activity in the *in vivo* and/or *in vitro* system of interest, and its subsequent effect then assayed.

5   The information obtained through such characterisations can suggest relevant methods for the treatment or control of nervous system disorders. For example, relevant treatment can include a modulation of gene expression and/or gene product activity. Characterisation procedures such as those described herein 10   can indicate whether such modulation should be positive or negative. As used herein, "positive modulation" refers to an increase in gene expression or activity of the gene or gene product of interest. "Negative modulation", as used herein, refers to a decrease in gene expression or 15   activity.

In vitro systems can be designed to identify compounds capable of binding the PKCγ mutant or wild-type gene products of the invention. Compounds identified, for example, could be useful in modulating the activity of wild 20   type or mutant PKCγ gene products, could be useful in elaborating the biological function of the PKCγ gene products, or could disrupt or enhance normal PKCγ gene product interactions, for example, the activators or inhibitors of PKC type I protein as disclosed in Keenan et 25   al, 1997, FEBS Letters, 415 pp101 - 108. Such compounds may be investigated for their use in treating or alleviating motor impairment and/or dopaminergic cell

degeneration disorders.

These and other aspects of the invention shall now be further described, by way of example only, and with reference to the accompany figures which show:

5   **Figure 1** is a diagram illustrating the region of rat genome selected to contain recombination events and genetic markers used to establish panels of backcross progeny recombinant in the interval containing the mutant PKCγ gene hereinafter referred to as nng3. The three backcrosses (BN

10   x NNG3) F1 x NNG3, (F344 x NNG3) F1 x NNG3 and (DA x NNG3) F1 x NNG3 are labelled BN, F344 and DA respectively;

     Figure 2 is a sequence alignment of sequence obtained from the PKCγ gene in rat strains NNG3 and AS. Sequences are

15   aligned to the rat (Rattus rattus) mRNA sequence obtained from NCBI (Accession number: X07287). The point mutation identified within the nng3 sequence is shown in bold and underlined at nucleotide 841. The translation start site is shown underlined, and the microsatellite defining the

20   marker R158 (within the 3'UTR) is shown in italics. The primers defining the marker R158 are shown underlined and indicated by arrows. The normal translation stop site is shown at nucleotide 2093, in bold and underlined;

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Figure 3 is an alignment of PKCγ DNA sequence as illustrated in Figure 2 with the predicted amino acid sequence. The sequence illustrated is from the rat strain *Rattus rattus*, and the nucleotide conversion seen in the sequence illustrated in Figure 2 is given in bold, below the sequence for amino acid number 281. The normal translational start and stop codons are shown in bold. The site at which a codon encoding the amino acid Glu is changed by the mutation to a STOP codon, resulting in a polypeptide terminator, is also indicated and is shown in bold;

Figures 4 (a) and (b) are immunocytochemistry stainings of rat brains with anti-PKCγ antibody. Figure 4(a) illustrates AS control rat brain stained with PKCγ, with the Purkinje cell layers 10 and granule cell layers 15 indicated. Figure 4(b) illustrates NNG3 rat brain stained with anti-PKCγ antibody;

Figure 5 illustrates a western blot of total brain proteins from the NNG3 and AS strains probed with an anti-PKCγ antibody. The lane marker (M) contains a BENCHMARK™ prestained protein ladder (GibcoBRL) and the sizes of the bands are indicated on the left-hand side. The lanes marked AS and NNG3 contain proteins isolated from brain tissue from AS and NNG3 rat strains, respectively; and

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Figure 6 illustrates an in situ hybridisation examination of the PKCγ mRNA transcript in the rat strains NNG3 and AS, as labelled. The brain sections from each strain were consecutive and all sections were probed simultaneously.

5   **Figure 7** illustrates an alignment of the AS Clone 17 sequence (bottom) and sequence from rat mRNA protein kinase C gamma from the NCBI database (Accession number: X07287) (top). A black dot between the sequences indicates identical bases. The start and stop codons are indicated

10   in bold.

Figure 8 illustrates an alignment of sequence obtained from the clone (Clone 28) of human full length cDNA, aligned to sequences obtained from the NCBI database (Accession

15   numbers: Z15114, H.sapiens mRNA for protein kinase C gamma (partial) and M13977, Human (clone lambda-hPKC-gamma6) protein kinase C-gamma (PRKCG).

Figure 9 is a comparison of the DNA sequence of rat PKCγ

20   cDNA and NNG3 cDNA. The rat PKCγ sequence is on the top, the NNG3 sequence is on the bottom. The start and stop codons are shown in bold. There is a base error in the NNG3 sequence at base 1281, after the stop codon.

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Figure 10 is a comparison of the amino acid sequences of the translated PKCγ and NG3 cDNAs. The NNG3 sequence is shown on top whilst the rat PKCγ sequence is shown below.

Figure 11: illustrates a Western blot of rat brain protein. (Primary antibody: RBI polyclonal PKC gamma at a dilution of 1:1000, Secondary antibody dilution: 1:1000).

Figure 12 illustrates the results of the inclined ramp test from two independent experiments. Plank 1 is 120mm wide, plank 2 is 100mm, plank 3 is 70mm wide, plank 4 is 50mm wide, plank 5 is 20mm wide and plank 6 is 10mm wide. The control animals were non-injected. The vector animal received pRcCMV2 without an insert. DNA refers to an injection of ratPKCγ in pRcCMV2. 1μg of vector or DNA was delivered in PEI at a charge ratio of either 1:100 or 1:1000, as indicated.

Experiment 1 - Genetic fine mapping of the nng3 mutation by genotyping of backcross progeny with R158

The present inventors applied genetic mapping techniques in order to ascertain the genotypic variation displayed in the AS/AGU strain using the process of "positional cloning" (Collins, 1992, Nature Genetics, 1, 3 - 6). Application of this approach relied upon initially determining the chromosomal localisation of the gene by demonstrating linkage to known marker genes. This was followed by additional fine mapping to narrow down the

genetic region containing the gene, followed by either
sequencing of the region or selection of mRNA transcripts
from the region.

Genetic linkage is a direct consequence of the
physical linkage of two or more genes with the same pair of
DNA molecules that define a particular set of chromosome
homologs within the diploid genome (Silver, 1995, Mouse
Genetics: Concepts and Applications, Oxford University
Press). Generally, crossing over occurs at random sites
along all the chromosomes in the mammalian genome. A
direct consequence of this randomness is that the further
apart two linked loci are from each other, the more likely
it is that a crossover event will occur some where within
the length of chromosome which lies between them (Silver,
1995). Thus, the frequency of recombination provides a
relative estimate of the genetic distance between a known
marker gene and a previously unknown gene.

Three backcrosses were established with the NNG3
strain and strains F344, BN and DA (Festing, 1979 Inbred
Strains in Biomedical Research, London : The MacMillan
Press Ltd.) to allow mapping of the nng3 gene. The F344,
BN and DA rat strains were chosen as they exhibited the
highest variation within microsatellite sequences when
compared with the NNG3 strain (Shiels et al, 1995,
Mammalian Genome, 6, 214 - 215). Microsatellite sequences
are defined as tandem repeats of simple dinucleotide or
other DNA sequences which occur in allelic forms of various
lengths. They are considered convenient genetic markers

and are examined by assessing the length of a short
polymerase chain reaction product containing the
microsatellite using gel electrophoresis.

To establish a backcross, each strain in question (DA,
F344 and BN) was crossed to the NNG3 strain and the
5   resulting heterozygous F1 progeny were backcrossed to the
NNG3 strain. The resulting backcross progeny were then
genotyped to identify if a cross-over event had occurred
between the gene of interest and any genetic marker. This
allowed positioning of the gene to within a particular
10   chromosome or sub-chromosomal region.

In total 3188 backcross progeny were produced from the
three backcrosses. A whole genome scan was carried out
with 73 microsatellite markers involving genotyping at
least one informative marker per rat chromosome. All of
15   these markers were assayed for linkage to the nng3
mutation. Genetic linkage was observed with the marker R33
(Serikawa et al, 1992, Genetics, 131, 701 - 721), which was
localised to chromosome 1 and mapped approximately 30 cM
from the nng3 gene. A range of markers from within this
20   chromosomal region were then used to genotype all of the
backcross progeny. The closest marker loci bracketing the
nng3 mutation were chosen to establish a chromosomal
interval within which to carry out precise mapping. This
interval was different for each backcross, as shown in
25   Figure 1. This allowed fine mapping of the region and
identified recombination events between markers. The
markers used are shown in Figure 1 for the three

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backcrosses (BN X NNG3) F1 X NNG3, (F344 X NNG3) F1 X NNG3

and (DA X NNG3) F1 X NNG3.

All progeny identified to have recombination events

within the interval under investigation were genotyped

using the genetic marker R158 (Serikawa et al, 1992). The

5   genetic marker R158 consists of a pair of PCR primers which

amplify a $(CA)_{26}$ microsatellite repeat from the 3'UTR of the

PKCγ gene. The three rat strains BN, F344 and DA were

shown to be informative for R158, that is, the length of

the microsatellite was shown to vary between strains, when

10   compared to the NNG3 rat strain. The genotyping was

carried out by PCR, as described in the following

experimental section, on genomic DNA. The PCR products

were then resolved by gel electrophoresis either on 6%

acrylamide or on 4% metaphor (Flowgen) agarose gels.

15   From this experiment (n=3188), no animals were

observed to contain a recombination event between the nng3

mutation and the marker R158. This positioned the nng3

mutation 0 ± < 0.06 cM from the genetic marker R158. In

the mouse, 0.06 cM corresponds to approximately 60 kb of

20   chromosome DNA length. These mapping experiments show that

the nng3 mutation (and gene) is very close to the R158

microsatellite, which is itself within the gene PKCγ

encoding the type I isoform of protein kinase C. Thus, the

nng3 gene is very likely to be PKCγ itself or an

25   immediately adjacent gene.

Experiment 2 - Demonstration of a DNA sequence difference between the allelic forms of the PKCγ gene in the strain and the parent AS strain

The genetic mapping evidence implicated the PKCγ gene as the location of the nng3 mutation. If this implication

5   was correct, a DNA sequence difference must have existed between the allelic forms of the PKCγ gene in the NNG3 strain and in the AS strain from which the NNG3 strain arose by spontaneous mutation. Thus, the following experiment was performed to provide evidence of the

10   sequence difference.

RNA was isolated from 12 month old rats from both the mutant (NNG3) and control (AS) strains. RNA was isolated using TRI REAGENT™ (Sigma) as per the protocol supplied by the manufacturer. RNA was isolated from 1g of brain tissue

15   and homogenised in 10 ml of TRI REAGENT™. $1\mu g$ of RNA was then used to synthesise cDNA using the Oligo $(dT)_{12-18}$ Superscript™ Preamplification System for First Strand cDNA Synthesis kit from Gibco BRL. 50ng of cDNA was then used as template in a PCR reaction. The PCR reaction was

20   carried out using 1 unit of *Taq* DNA polymerase (Promega) in a reaction containing 1x magnesium free Thermo buffer, 1mM magnesium chloride (all supplied with *Taq* polymerase from Promega), $125\mu M$ dNTPs (Promega) and forward and reverse primers at $5ng/\mu l$ each. A hot start was always performed

25   in the PCR reaction. The sequences of all PCR primers used are given in Table 1, along with the PCR reactions in which they were used.

Primer name	Primer sequence 5' to 3'	Utility
Neup113 Forward	GCTACTCAAGGCTCCTGTGGATGG	RT-PCR
Neup114 Reverse	ATGAGATTACATGACGGGCACA	RT-PCR
Neup120 Forward	CAAGGCTCCTGTGGATGGATGG	Genomic PCR
Neup120 Reverse	GCTGCAGTTGTCAGCATCGGC	Genomic PCR

Table 1 - PCR primers utilised. Sequences of PCR primers used are given along with the reactions in which they were used.

The PCR parameters used for *Taq* DNA polymerase were as follows:

<u>113 Forward/114 Reverse</u>　　　　<u>120 Forward + Reverse</u>

99°C - 3 mins　　　　　　　　　　99°C - 10 mins

80°C - Taq added　　　　　　　　80°C - Taq added

Followed by 35 cycles of:　　　Followed by 30 cycles of:

94°C - 15 secs　　　　　　　　　94°C - 15 secs

55°C - 30 secs　　　　　　　　　55°C - 30 secs

72°C - 1 min. Followed by:　　72°C - 30 secs. Followed by:

72°C for 10 mins.　　　　　　　72°C - 10 mins.

PCR products were then resolved on a 2% agarose gel (Boehringer Mannheim) and then extracted from the gel using the Qiaquick™ gel extraction kit prior to sequencing.

Sequencing of the PCR products was carried out on the ABI 373 stretch automatic sequencer using the Big Dye terminator chemistry (Perkin Elmer). 3.2 pmoles of a primer used in the PCR reaction were used for sequencing.

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PCR reactions were also carried out on rat genomic DNA isolated from rat spleen taken from both rat strains. DNA was isolated using the Pure Gene DNA Isolation kit (Gentra). PCR reactions were carried out as before containing 100 ng of genomic DNA as template. The initial

5   denaturation step of the PCR reaction was also increased to 10 minutes.

PCR reactions were also carried out using proof-reading DNA polymerase enzymes to eradicate errors during DNA polymerisation. The enzymes and modifications of the

10   PCR reactions were as follows: *Tli* polymerase (Promega) reactions were carried out with 1.25 units of the enzyme, which extends at 74°C. The High Fidelity (HF) kit from Clontech has an automatic hot start and anneals and extends concurrently at 68°C for 3 minutes.

15   Three different populations of cDNA were synthesised and PCR products obtained from these sequences (a summary of experimental results is given in Table 2). All PCR products sequenced exhibited a G nucleotide in the AS rat strain at position 841 and a T nucleotide at the same

20   position in the NNG3 rat strain, as shown in Figure 2. This transversion mutation creates a new termination or stop codon for translation of PKCγ, resulting in a prematurely terminated protein (Figure 3). As the kinase active domain of the protein is lost, this will result in

25   a loss of ability to phosphorylate the target protein substrates, leading to a cascade of biological consequences. This proposed loss of function appears to be

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in agreement with the recessive nature of the mutation.

Experiment no.	Template	PCR Enzyme	Summary
1	cDNA: 1	Taq	G at position 841 in AS and T at 841 in NNG3
2 A: B:	cDNA: 2 cDNA: 1	Taq Taq	G at position 841 in AS and T at 841 in NNG3
3	cDNA: 2	Tli (proof-reading)	G at position 841 in AS and T at 841 in NNG3
4	cDNA: 2	HF-kit (proof-reading)	G at position 841 in AS and T at 841 in NNG3
5	cDNA: 3	HF-kit	G at position 841 in AS and T at 841 in NNG3
6	Genomic DNA	Taq	G at position 841 in AS and T at 841 in NNG3
7	Genomic DNA	Tli	G at position 841 in AS and T at 841 in NNG3

Table 2 - Summary of sequencing experiments carried out.
The table details the enzymes and templates used in PCR
reactions carried out for sequencing. The nucleotide
number referred to in the table (841) is taken from Figure
2.

These mapping and sequencing results demonstrate that
a sequence difference does appear to occur between the NNG3
and AS strains. It is conceivable that this base change (G
to T at position 841, Figure 2) in the NNG3 strain gives
rise to the phenotype observed in this strain.

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Experiment 3 - Detection of PKC gamma by immunocytochemistry

It was postulated that the stop codon in the PKCγ gene in the NNG3 strain led to premature termination of the PKC type I polypeptide.   This would result in the protein

5   having no kinase activity.   The binding of antibodies raised against the carboxy terminal portion of the PKC type I protein (Boehringer Mannheim) to brains isolated from the NNG3 strain were thus investigated.

PKC gamma was detected by immunocytochemistry using

10   standard protocols with the following modifications: rat brains were taken from 9 months old male rats and were fixed overnight in 4% paraformaldehyde.   Brains were then trimmed, placed on a block and sections cut at 50 microns using a vibratome.   Paired sections were placed into

15   blocking serum (10% NGS - normal goat serum) and placed on shaker for 1 hour at room temperature.   Sections were then placed into primary antibody (rabbit anti-peptide antibody generated using a synthetic peptide corresponding to amino acids 306 - 318 of rat PKCγ, 1:100 - 1:2000, Boehringer

20   Mannheim) overnight at 4°C.   Slides were then washed three times in PBS for 5 mins each.   Sections were then placed into secondary antibody (biotinylated sheep anti-rabbit IgG antibody, Vector) for 1 hour.   Sections were again washed three times in PBS (5 mins each).   Vecta stain ABC complex

25   (Vector) was then added to the sections for 1 hour and placed on a shaker.   Sections were again washed three times in PBS (5 mins each) and then washed once in PB for 5 mins.

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3,3'Diaminobenzidine tetrahydrochloride (DAB) was applied
to the sections for 5-10 mins. Sections were washed twice
in PB (5 mins each) and the sections finally dehydrated,
cleared and mounted.

Figures 4(a) and (b) illustrate the difference between
the NNG3 strain and the AS parent strain, from which NNG3
is derived.

A very striking depletion of PKCγ positive cells in
Purkinje cells of the NNG3 strain was observed when
compared to an age matched AS control. The Purkinje cell
layer is a region where PKCγ is predominantly expressed
when compared to other PKC isoforms.

These results are consistent with a loss of the
carboxy-terminal part or all of the PKC type I protein.

**Experiment 4 - Western blot detection of Type I PKC in rat
brain protein extracts by antibody hybridisation**

A Western blot experiment was carried out to determine
the level of expression of the Type I PKC protein in brains
from the rat strains AS and NNG3. Specifically, antibodies
raised against a peptide located in the carboxy terminal to
the truncation site in the PKCγ type I protein of the NNG3
strain were used in order to confirm the lack of expression
of this region of the protein.

Total brain proteins were extracted from male, 9 month
old rats from both the AS and NNG3 stains. The proteins
were isolated from 0.2g of brain tissue using TRI-REAGENT™
(Sigma) and suspended in 2% SDS. 50µg of total proteins

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were resolved on a 10% SDS-PAGE (polyacrylamide gel electrophoresis) gel at 200V for 45 minutes. The proteins were then transferred to nitrocellulose (Amersham Life Science) in a wet Western blotter at 30V overnight. The nitrocellulose was then blocked for 1 hour at room

5   temperature, in a solution of Tris-buffered saline (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) containing 0.05% Tween 20 (TBST) and 1% bovine serum albumin (BSA). The nitrocellulose was then incubated in TBST plus 1% BSA containing 2 μg/ml anti-PKCγ (Rabbit anti-peptide antibody

10 generated using a synthetic peptide corresponding to amino acids 306-318 of PKCγ, GibcoBRL). The blot was then washed 3 times in TBST for 10 minutes each. The blot was incubated for one hour at room temperature in TBST containing anti-rabbit Ig, peroxidase-linked species-

15 specific whole antibody (from donkey, Amersham Life Science) at a dilution of 1/1000. The blot was then washed as detailed above. After washing the blot was incubated with ECL Western blotting detection reagents (Amersham Pharmacia biotech) and then exposed to autoradiography film

20 (Fuji XR).

This experiment was performed on proteins extracted from the NNG3 strain and the control rat strain (AS). The results obtained are illustrated in Figure 5. A band of the predicted size (80 kDa) was obtained from the AS strain

25 protein extract, whereas no signal was obtained in the NNG3 strain protein extract. The anti-PKCγ antibody recognises an epitope corresponding to amino acids 306-318 of the

protein.   In NNG3 it is postulated that a truncated protein
is produced which terminates at amino acid 281; therefore,
these results illustrate that the epitope for antibody
binding is not present in the NNG3 protein, as predicted.

5   **Experiment 5 - _In situ_ hybridisation investigation of the
     PKCγ mRNA transcript in AS and NNG3 rats**

          An _in situ_ hybridisation experiment was carried out to
     determine the level of expression of the mRNA encoding Type
     I PKC in the rat strains AS and NNG3.

10   An antisense oligonucleotide probe was designed to the
     3' region of the PKCγ MRNA (nucleotides 2085-2326, numbers
     taken from Figure 2) and was synthesised and purified by
     HPLC (GibcoBRL).   The sequence of the oligonucleotide was
     as follows:

15   5' GCA CTG GGA ACA CCT AGC GGC AGC AGA TGA GAT TAC ATG ACG
     3'

          Whole brains were taken from 8 month old AS and NNG3
     rats.   These brains were mounted and sectioned horizontally
     in 13 micron sections and the sections thaw-mounted onto
20   poly-L-lysine treated microscope slides.   The sections were
     then fixed in 4% paraformaldehyde in 1x PBS (phosphate
     buffered   saline,   all   solutions   were   made   with
     (diethylpyrocarbonate-treated water) on ice for 5 minutes,
     followed by PBS for 2 minutes, then dehydrated in 70%
25   ethanol for 2 minutes, 95% ethanol for 5 minutes and stored
     in 100% ethanol at 4°C   until required.

The *in situ* hybridisation probes are labelled and prepared for hybridisation in the following way:

40ng of the oligonucleotide was labelled in a reaction volume of 12.5μl made up in DEPC-water containing 1x reaction buffer (supplied with enzyme), 25μCi of S^{35} α-dATP and 36 units of Terminal deoxynucleotidyl transferase (TdT, FPLC pure, Pharmacia). The reaction was then incubated at 37°C for 1 hour. Purification columns were constructed in 1 ml plastic syringes with GF/C filter paper, cut with a No. 2 cork borer, placed at the bottom of the syringe and the syringe packed with G50 Sephadex (Pharmacia). The column was then centrifuged at 2,000 rpm for 1 min to pack the Sephadex. 87.5 μl of DEPC-treated water was then added to the probe to make to 100 μl. The probe was added to the column and centrifuged for a further 1 minute at 2,000 rpm in a 1.5 ml centrifuge tube for collection. The eluant was then collected and the volume measured. The specific activity of the probe was determined by placing 2 μl of the eluted probe in a scintillation vial and adding 5 ml of scintillant. The specific activity of the probe was then measured in a scintillation counter uwing the Tritium channel. The probe was then standardised so as produce 2 x 10^3 disintegration's per minute/ml of hybridisation mix 50% formamide, 4 x SSC, 10% dextran sulphate, 5 x Denardt's, 200μg/ml acid-alkali cleared salmon sperm DNA, 100μg/ml long chain polyadenylic acid, 120μg/ml heparin, 25mm sodium phosphate, pH7, 1mm pyrophosphate, then DDT added to a final concentration of 20mm. A 100 times excess

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of cold oligonucleotide was also added to the control

hybridisation mix.

Serial sections were selected for hybridisation so as

to be pair-matched and spread throughout the brain. The

sections were covered with 200 μl of the appropriate

5   hybridisation mix and then covered with a parafilm

coverslip. The slides were then incubated overnight at

42°C. The following day the coverslips were floated off in

1 x SSC at room temperature and the sections were washed

twice in a shaking water-bath at 55°C in 1x SSC containing

10   4 mM DTT for 30 minutes. The sections were then dehydrated

through 1x SSC for 30 seconds, 0.1x SSC for 45 seconds,

then 70% ethanol for 2 minutes and finally 100% ethanol for

5 minutes. The slides were then allowed to air dry. Once

dry the slides were taped to 3MM filter paper and exposed

15   to autoradiography film (Kodak Bio-max MR film, single

coated) at room temperature for 1 week.

The results of the *in situ* hybridisation experiment

are illustrated in Figure 6. It is evident that the level

of PKCγ mRNA in the NNG3 strain appears to be altered when

20   compared to age-matched control brains from the AS strain.

Experiment 6 - Isolation of PKCγ cDNAs from rat and human

Full length cDNA clones were isolated for the PKCγ

gene, by reverse transcriptase-polymerase chain reaction

25   (RT-PCR) amplification, from RNA isolated from both the rat

strain Albino Swiss (AS) and human. The brain was removed

from a 12 month old male AS rat after decapitation and 1g

of tissue removed. Total RNA was isolated from the tissue using TRI REAGENT™ (Sigma, Poole, Dorset) as per the protocol supplied by the manufacturer. Human brain RNA from a normal individual was obtained from Stratagene. 1 μg of RNA from each was then used to synthesise cDNA using the

5   Oligo (dt)$_{12-18}$ Superscript™ Preamplification System for First Strand cDNA synthesis kit from Gibco BRL (Inchinnan Business Park, Paisley). 50 ng of cDNA was then used as template in PCR reactions. All PCR reactions were carried out using the Clontech Advantage®-HF PCR kit as per the

10   manufacturers instructions (Basingstoke, Hampshire). The full length AS cDNA was amplified using the primers Neup 118F 5' -CCT TCC GAT CTC AGA GTC TGC GG- 3' and K3R 5'-TTC TAC AAC TGA AGT GGA GG-3' (PCR parameters: 94°C for 15 seconds, 25 cycles of 94°C for 10 seconds, 64°C for 4

15   minutes, followed by 68°C for 3 minutes). The full length human cDNA was amplified using the primers Neup 144F 5'- TGA TCC TTC GAG TCT CCA GC-3' and Neup 144R 5'- ACG TTG GGG ACA CCT AGT GG-3' (PCR parameters: 94°C for 15 seconds, 25 cycles of 94°C for 10 secs, 68°C for 3 minutes, followed by

20   68°C for 3 minutes). The amplified fragments were cloned using the Zero Blunt® TOPO® PCR cloning kit (Invitrogen, CH Groningen, Netherlands).

Sequence analysis was then carried out on the clones to investigate any errors introduced during PCR

25   amplification. Sequencing reactions were carried out using the BigDye terminator kit (PE Biosystems, Birchwood science park, Warrington) in the presence of 3.2 pmoles of a primer

used in the PCR reaction. The reactions were carried out

in a Perkin Elmer GeneAmp PCR system 9600 (Perkin Elmer,

Birchwood science park, Warrington) as follows: 96°C for 2

minutes, 25 cycles of 94°C for 10 seconds, 50°C for 5

seconds and 60°C for 4 minutes. The sequencing reactions

5   were resolved on an ABI 373 stretch automatic DNA

sequencer. Sequence data was analysed using Seqed software

(PE Biosystems).

Sequencing results demonstrated that there was one

base change identified in the AS sequence when compared to

10   the sequence lodged in the database (NCBI Accession number:

X07287). This base change CAA->CAG (base 201, Figure 7)

did not change the amino acid sequence and may simply

indicate a polymorphism. There is also a G which is not

present in AS when compared to the mRNA protein kinase C

15   gamma (base 2236). This base deletion occurs after the

stop codon and may also represent a species difference.

Some differences were also observed in the human cDNA

clone when compared to the two sequences lodged in the NCBI

database (Table 3 and Figure 8), but again these did not

20   change the amino acid and may indicate polymorphism.

Indeed one of these polymorphisms had previously been

described by Al-maghtheh, et.al., (Al-Maghtheh, M.,

Vithana, E.N., Inglehearn, C.F., Moore, T., Bird, A.C. and

Bhattacharya, S.A. (1998). Segregation of a PRKCG mutation

25   in two RP11 families. American Journal of Human Genetics,

62, 1248-1251), AAT189AAC. The two sequences lodged in the

database were also polymorphic at this base.

Base number (Fig 8)	Base Change	Amino acid Conserved
56	TTC->TTT	Phe
392	TGT->TGC	Cys
395	TCT->TCC	Ser
444	TCT->TCC	Ser
567	AAT->AAC	Asn (Al-maghtheh, 1998 AAT189AAC)
873	GCT->GCC	Ala

Table 3 - Base changes identified within Human clone 28 when compared to the sequence lodged in the NCBI database (Accession numbers: Z15114, H.sapiens mRNA for protein kinase C gamma (partial) and M13977, Human (clone lambda-hPKC-gamma6) protein kinase C-gamma (PRKCG).

Experiment 7:   Cloning of the NNG3 PKCγ cDNA

PCR of the full length cDNA had been attempted, but failed. Therefore, PCR amplification of the 5' and 3' ends of the NNG3 cDNA, in two separate reactions was performed.

5' rapid amplification of cDNA ends (RACE)

Total RNA from NNG3 rat brain was prepared using Tri-reagent (Sigma) according to manufacturers instructions. 1mg of this total RNA was used with the 5' RACE system (Gibco BRL). The protocol supplied with the kit was

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followed. Gene specific primer 104R (5' - CTTA
AACTTGCCCAGTTGCT - 3') was used as the GSP1. This
corresponds to the antisense sequence between bases 1480
and 1500 of the PKCγ cDNA (see figs 7 and 9). The PCR
reaction was conducted using 0.4 μl of the high fidelity

5   enzyme, Expand (Roche Diagnostics Ltd, Lewes, East Sussex),
with 1μl 10mM dNTPs, 5μl template cDNA, 5μl 10 x expand
buffer, 3μl 25 mM MgCl$_2$, 1μl of 10μM abridged anchor primer
(5'-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG - 3') and 1μl of
1μM GSP1. The following conditions were employed for the

10   PCR, using a Perkin Elmer GeneAmp PCR system 9600: 94°C for
2 minutes then 30 cycles of 94°C for 15 seconds, 50°C for 30
seconds and 72°C for 2 minutes and finally 7 minutes at
72°C.

The product of this PCR was a smear. Therefore the DNA

15   was diluted 1:500 in T/E (10mM Tris pH 8.0, 0.1 mM EDTA)
and a second round of PCR was performed. The same reaction
conditions as above were used, but the abridged anchor
primer was replaced with the gene specific primer 118F (5'
- CCTTCCGATCTCAGACT - 3').

20   A product of the expected size (1.3kB) was obtained
from this round of PCR and it was directly cloned into the
vector pCRbluntIITOPO using the Zero Blunt® TOPO® PCR
cloning kit (Invitrogen) and manufacturers directions. It
was named NNG3 5/12.

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3' RACE

Total RNA from NNG3 rat brain was prepared using Tri-
reagent (Sigma) according to manufacturers instructions.
1mg of this total RNA was used with the 3' RACE system
(Gibco BRL). The protocol supplied with the kit was
5   followed.

The PCR reaction was performed using the above
conditions and components but replacing the primers with
the universal amplification primer
(5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC - 3') and the gene
10   specific primer 113F (5' - GCTACTCAAGGCTCCTGTGGATGG - 3')
corresponding to the region 793 to 816 bases of the PKCγ
cDNA (see figs 7 and 9).

Again the product of this PCR was a smear. Therefore
the DNA was diluted 1:500 in T/E and a second round of PCR
15   was performed. The same reaction conditions as above were
used, but the universal amplification primer was replaced
with a second gene specific primer 114R (5' -
TGAGATTACATGACGGGCACA - 3') corresponding to the region
antisense between bases 2077 and 2096 of the PKCγ cDNA (see
20   figs 7 and 9).

A product of the expected size (1.3kB) was obtained
from this round of PCR and it was directly cloned into the
vector pCRbluntIITOPO using the Zero Blunt® TOPO® PCR
cloning kit (Invitrogen) and following manufacturers
25   directions. It was named NNG3 3/9.

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The two NNG3 clones provide overlapping sequence between bases 797 and 1357. A single BamHI restriction digest site at 1112 bases was therefore used to join the two fragments together.

NNG3 5/12 and NNG3 3/9 were digested with HindIII (Promega) and BamHI (Promega) to release the 5' NNG3 fragment from both plasmids. The NNG3 5' fragments was purified from the NNG3 5/12 digest whilst the plasmid was purified from the NNG3 3/9 digest. Agarose gel purification was performed using a Qiaquick Gel Extraction kit (Qiagen).

The fragment purified from NNG3 5/12 could then be ligated into NNG3 3/9 to give the full length NNG3 cDNA. The DNA was then sequenced to check assembly and to ensure that no mutations had been introduced by PCR.

The two proteins were then translated using Seqed software (PE biosystems) and it could be seen that the NNG3 protein is terminated at amino acid 280 (see Figure 10).

Experiment 8 – Western blot of PKCγ

Brain protein extracts from normal AS rats and NNG3 rats were prepared by homogenising 500mg of tissue in 2.5ml of ice cold 0.2 M potassium phosphate buffer, pH 7.6. The homogenates were centrifuged at 1000g for 15 minutes at 4°C. The supernatant from each sample was carefully removed with a syringe and stored at -20°C in aliquots.

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The protein concentration of each sample was measured using a bicinchinic acid protein assay kit (Sigma). 30μg of each protein sample, combined with an equal volume of sample buffer (0.0625M Tris, pH 6.8, 2% sodium dodecyl sulphate, 10% glycerol, 5% β-mercaptoethanol, 0.001% bromophenol blue), was then loaded onto a 10% polyacrylamide gel (28.2:0.8 acrylamide:bisacrylamide, 0.375 M Tris, pH 8.8, 0.1% sodium dodecyl sulphate) with a 3% stacking gel (28.2:0.8 acrylamide:bisacrylamide, 0.125 M Tris, pH 6.8, 0.1% sodium dodecyl sulphate). Prestained Benchmark protein standards (Gibco BRL) were loaded alongside the protein samples to provide a reference for the molecular weight of the protein of interest. Running buffer (0.025 M Tris (base), 0.192 M glycine, 0.1% sodium dodecyl sulphate) was added to the mini-gel apparatus (Biorad, Hemel Hempstead, Hertfordshire) and a voltage of 200V was placed across the gel, using a Biorad Power Pack 300 electrophoresis power supply unit.

After 45 minutes the dye front had reached the bottom of the gel, indicating that the electrophoresis of the gel was complete. The gel apparatus was dismantled and the gel was placed in a western blotting apparatus (Biorad) against a piece of PVDF membrane (Roche). The proteins were transferred onto the membrane using Towbin buffer (12.11.g Tris (base), 57.6 g Glycine, 800ml methanol, 1.2 ml hydrochloric acid in a volume of 4 1) and a voltage of 40v, applied over night.

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The membrane, with bound proteins, was removed from the apparatus and submerged in blocking solution (10% (w/v) dried milk in PBS-T (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 adjusted to pH 7.4 and made up to 1l, with 0.1% tween 80) for 30 minutes. A second wash of 30 minutes was then performed.

Antibody, specific for PKCγ (epitope amino acids 684-697)(RBI, Sigma, Poole, Dorset) was diluted, in blocking solution, to a concentration of 1:1000 and the membrane was placed in this for three hours, with gentle agitation. After incubation the membrane was washed three times in blocking solution, ten minutes each wash. HRP-labelled secondary antibody, specific for rabbit (Amersham, Buckinghamshire), diluted to 1:1000 in blocking solution, was then poured over the membrane and incubation allowed to proceed for 1 hour, with gentle agitation. The membrane was finally washed three times in PBS-T, for ten minutes each wash.

Detection reagent was made up using an equal volume of ECL reagent 1 and 2 (Amersham). The membrane was incubated in this for 1 minute, then wrapped in Saran Wrap (Dow, Germany) and exposed to X-ray film (Konica, Tokyo, Japan) for five minutes before being developed using an X2 processor (Genetic Research Instrumentation, Dunmore, Essex).

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The Western blot shown inFigure 11 demonstrates that
full-length PKCγ is not expressed in the brain of the NNG3
rat. However, it is not possible to determine whether a
truncated form of the protein is expressed, or whether PKCγ
is missing in its entirety, from the brain of the NNG3 rat.

Experiment 9 - Microinjection of PKC γ to allow expression in the brain

This work follows a protocol originally reported in
Martes et.al. (Martres, M-P., Demeneix, B., Hanoun, N.,
Hamon, M. And Giros, B. (1998). Up-and down-expression of
the dopamine transporter by plasmid DNA transfer in the rat
brain. European Journal of Neurosciences, 10, 3607-3616).
The full length AS cDNA isolated, as described previously,
was removed from the TOPO vector by restriction digest
using HindIII and XbaI and cloned into the pRc/CMV2 vector
(Invitrogen). DNA was then isolated from this clone using
the EndoFree Plasmid Giga kit (Qiagen, Crawley, West
Sussex), according to the manufactures protocol.

3 charge equivalents (taking into account that 1 µg of
DNA and 1 µl of 0.1 M PEI correspond, respectively, to 3
nmoles of phosphate and 100 nmoles of amine nitrogen) of
polyethylenimine (PEI) 25 kDa, (Aldrich, Poole, Dorset)
were combined with 1µg of DNA and vortexed for 30 seconds
prior to injection.

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Each animal was anaesthetised using Vetalar (ketamine hydrochloride; 100mg/ml; Code VM 14851/4009 Pharmacia & Upjohn Ltd, Animal Health Division, Crawley RH10 2LZ) and Rompun (Xylazine hydrochloride 2%; Bayer plc, Animal Health Business Group, Eastern Way, Bury St. Edmunds, Suffolk IP32

5   7AH) in a ratio of 2:1. Injections were given intraperitoneally at 1.1ml/kg.

Deep anaesthesia was established after 5-10 minutes using the 'pad pinching' technique where no reflexual withdrawing of the foot was observed when full anaesthesia

10   was established. A small area on the head was then shaved using an electric razor.

The animal was placed into ear bars on a stereotaxic frame (Kopf, Munich, Germany). The head was held in place using teeth bars and horizontally using a nose bar.

15   An incision was made using a scalpel blade and the skin was held back from the skull using skin retractors (Merck Ltd, Hunter Boulevard, Magna Park, Lutterworth, Leicestershire LE17 4XN). The skull was scraped using a scalpel blade to reveal Bregma and the area was cleaned and

20   dried using a cotton bud. Bregma was marked using a pencil and confirmed independently prior to taking the Bregma co-ordinates. Lateral and anterior posterior co-ordinates were taken using the stereotaxic frame. The ventral co-ordinate was not taken at this point.

25   Co-ordinates were calculated, using the Paxinos and Watson stereotaxic brain atlas, and microinjection made at anterior/posterior (-3.8mm), lateral (+1.8mm) and ventral

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(-2.0mm) when compared with the co-ordinates of Bregma.

A 10μl Hamilton microsyringe needle containing 2μl of either pRc/CMV PKCγDNA in PEI, empty pRc/CMV vector in PEI or saline was placed directly over the brain area corresponding to these new co-ordinates. This was again
5   marked using a pencil and confirmed independently.

The tip of a drill head (1mm) was placed over the pencil mark and a hole was drilled into the skull. The area was again dried using a cotton bud. The microsyringe needle was then placed over the hole and lowered into it slowly.
10   The needle was then lowered into the brain to the appropriate depth according to the calculated ventral co-ordinate.

The needle was left in place for 2 minutes then 1μl solution was injected. The needle was left in place for a
15   further minute. At this time another 1μl of solution was injected. The needle was left in place for a further 2 minutes before being removed slowly.

The hole was then sealed with dental cement (Redifast 250ml Code 002363, Wright Health group Ltd, Kingsway West
20   Dundee), the animal was removed carefully from the stereotaxic frame and the incision was closed using standard cotton sutures.

The animal was then given a subcutaneous injection of 0.1ml Antisedan (atipamezole hydrochloride; 5mg/ml; Code VM
25   0057/4111, Pfizer Ltd; Ramsgate Road, Sandwich, Kent CT13 9LU) and allowed to recover before being placed back into its cage.

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The experiment was repeated with microinjection at co-ordinates of anterior/posterior (-3.8mm), lateral (+1.8mm) and ventral (-3.5mm) when compared with the co-ordinates of Bregma.

5   **Experiment 10 - Animal behaviour analysis**

Each animal was assessed 24hrs after surgery to establish that no adverse effects had resulted from the microinjection described above. Non-invasive locomotor tests were carried out after a further 24 hrs and repeated

10   every 48hrs until day seven. Three series of tests were carried out on each animal prior to decapitation on the seventh day.

The test chosen was the inclined ramp test.

15   **Inclined ramp test**

Six planks of wood were used ranging between 10-120mm. The six planks used were 10, 20, 50, 70, 90 and 120mm. Each rat was placed onto each plank assessed for its ability to maintain it's balance and walk down to the bottom of the

20   plank without falling off. This was carried out once. If the rat could do this, it received a score of 1 and if it fell off the plank it received a score of 0. This was repeated for all planks and was video-recorded.

These two experiments demonstrate that the

25   introduction of wild type PKCγ into the brain of NNG3 rats allows them to perform the inclined ramp test better than their uninjected counterparts. Preliminary evidence

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therefore suggests that PKCγ expression may help to rectify
the Parkinsonian phenotype.

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CLAIMS

1.   Use of a polynucleotide fragment comprising the PKCγ gene encoding the type 1 subtype of protein kinase C in the manufacture of a medicament for treating a neurodegenerative disorder.

2.   Use of a polynucleotide fragment according to claim 1 wherein the medicament is used to treat mammals.

3.   Use of a polynucleotide fragment according to claim 1 wherein the medicament is used to treat humans.

4.   Use of a polynucleotide fragment according to any preceding claim wherein the degenerative disorder is a degenerative disorder of the central nervous system.

5.   Use of a polynucleotide fragment according to claim 4 wherein the degenerative disorder of the central nervous system is Alzheimer's Disease.

6.   Use of a polynucleotide fragment according to claim 4 wherein the degenerative disorder of the central nervous system is associated with dopaminergic cell degeneration.

7.   Use of a polynucleotide fragment according to claim 4 wherein the degenerative disorder of the central nervous system is a neurodegenerative disorder associated with movement impairment.

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8. Use of a polynucleotide fragment according to either of claims 6 or 7 wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear

5 palsy, cortical-basal ganglionic (corticobasal) degeneration, vascular Parkinsonism and ballism.

9. Use of a polypeptide comprising protein kinase C type 1 in the manufacture of a medicament for treating a

10 neurodegenerative disorder.

10. Use of a polypeptide according to claim 9 wherein the medicament is used to treat mammals.

15 11. Use of a polypeptide according to claim 9 wherein the medicament is used to treat humans.

12. Use of a polypeptide according to any of claims 9 to 11 wherein a degenerative disorder is a degenerative

20 disorder of the central nervous system.

13. Use of a polypeptide according to claim 12 wherein the degenerative disorder of the central nervous system is Alzheimer's Disease.

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14. Use of a polypeptide according to claim 12 wherein the degenerative disorder of the central nervous system is associated with dopaminergic cell degeneration.

15. Use of a polypeptide according to claim 12 wherein the degenerative disorder of the central nervous system is a neurodegenerative disorder associated with movement impairment.

16. Use of a polypeptide according to either of claims 14 or 15 wherein the neurodegenerative disorder is selected from the group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear palsy, cortical-basal ganglionic (corticobasal) degeneration, vascular Parkinsonism and ballism.

17. Use of a polypeptide according to any of claims 9 to 16 wherein the polypeptide is synthetic.

18. A method of testing an animal thought to have a neurodegenerative disorder comprising detecting the presence of mutation in the PKCγ gene or its associated promoter.

19. A method of testing an animal thought to be predisposed to having a neurodegenerative disorder comprising detecting the presence of mutation in the PCKγ gene or its associated promoter.

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20. A method according to either of claims 18 or 19 wherein the animal is a mammal.

21. A method according to claim 20 wherein the mammal is a human.

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22. A method according to either of claims 18 and 19 wherein the neurodegenerative disorder is a degenerative disorder of the central nervous system.

10   23. A method according to claim 22 wherein the degenerative disorder of the central nervous system is Alzheimer's Disease.

24. A method according to claim 22 wherein the degenerative disorder of the central nervous system is

15   associated with dopaminergic cell degeneration.

25. A method according to claim 22 wherein the degenerative disorder of the central nervous systems is a

20   neurodegenerative disorder associated with movement impairments.

26. A method according to either of claims 24 or 25 wherein the neurodegenerative disorder is selected from the

25   group comprising Parkinson's Disease, Huntington's Disease/Chorea, Dementia with Lewy bodies, Multiple-system atrophy, Progressive supranuclear palsy, cortical-basal

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ganglionic (corticobasal) degeneration, vascular Parkinsonism
and ballism.

27. A method according to either of claims 18 or 19
wherein the mutation results in a truncated product from the

5   PKCγ gene being produced.

28. A method according to claim 27 wherein the mutation
occurs in the 5' half of the gene.

10   29. A method according to claim 28 wherein the mutation
is a point mutation at position 841 of the rat PKCγ gene or
a similar region of the PKCγ gene from another species.

30. A method according to either of claims 18 or 19
15   wherein detection of the presence of the mutation in the PKCγ
gene is achieved by detecting altered levels of the mRNA
transcripts or mRNA precursor.

31. A method according to either of claims 18 or 19
20   wherein the mutation in the PKCγ gene is detected using
antibodies raised to the truncated PKC type I polypeptide.

32. Use of a truncated PKCγ polynucleotide fragment for
promoting nervous system degeneration for the production of
25   animal models.

33. Use of a limited PKC type I polypeptide for promoting nervous system degeneration for the production of animal models.

34. Use of a PKCγ polynucleotide fragment encoding the
5   PKC type I polypeptide for preventing, delaying, treating or inhibiting degeneration of nervous system.

35. Use of a PKC type I polypeptide for preventing, delaying, treating or inhibiting degeneration of nervous
10 system.

36. A polynucleotide fragment encoding the PKC type I polypeptide for use in gene therapy.

15 37. Use of a PKCγ type I polypeptide for the identification of compounds for use in the treatment of neurodegenerative disorders.

38. An antibody specific for an epitope(s) located on
20 a truncated polypeptide produced from the PKCγ gene.

39. An antibody according to claim 38 wherein the epitope(s) is/are located in the C terminal half of the PKC type I polypeptide.

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40.   An antibody according to claim 42 wherein the C terminal half of the polypeptide begins at amino acid number 282 and ends at the C terminus of the native polypeptide.

41.   An antibody according to any of claims 38 to 40 wherein the antibody is a monoclonal antibody.

42.   The monoclonal antibody according to claim 41 wherein the monoclonal antibody has been humanised.

43.   Use of an antibody according to claims 38 - 42 for the manufacture of a medicament for preventing, delaying, treating or inhibiting degeneration of the nervous system.

44.   Use of an antibody according to claims 38 - 42 in a diagnostic assay for testing an human thought to have or be predisposed to having a neural degenerative disorder.

FIGURE 1
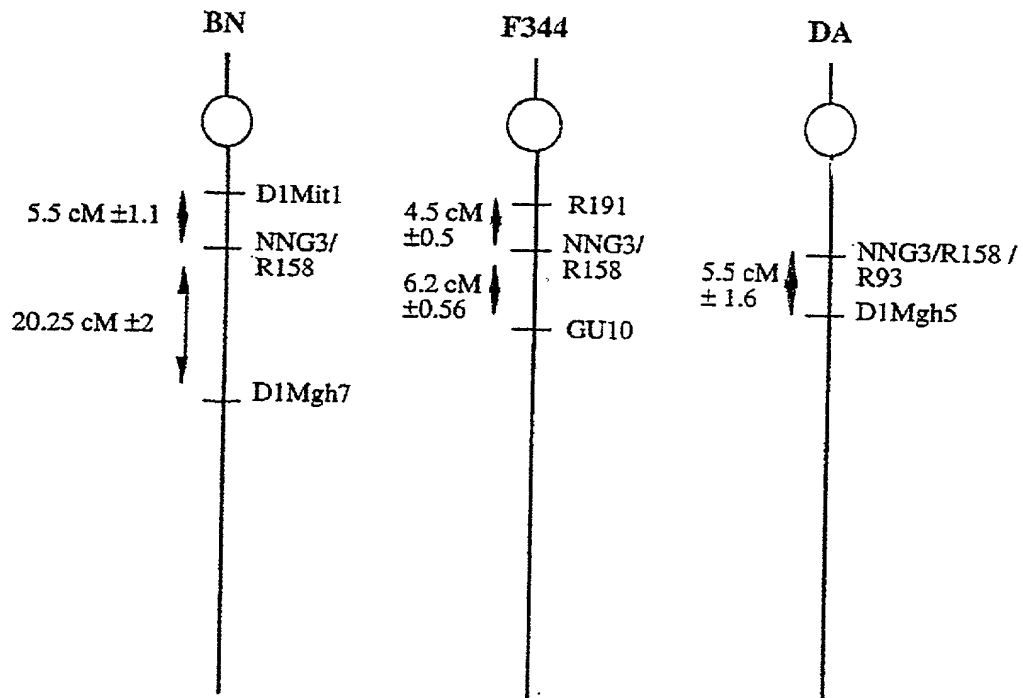
FIGURE 2

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            -140        -130        -120        -110        -100         -90
              |          |           |           |           |           |
Contig       TGCTTTCTGCCCTGCGCTGCGCACCGTTAGTGCCCTGCCCCTGTCCTTCCGATCTCAGAG
rat mRNA     TGCTTTCTGCCCTGCGCTGCGCACCGTTAGTGCCCTGCCCCTGTCCTTCCGATCTCAGAG
113/114 NNG3
113/114 AS


             -80         -70         -60         -50         -40         -30
              |           |           |           |           |           |
Contig       TCTGCGGAGTGCCCCTATCGCCGTCCACCTGTTTCCTCAGAAAAAAGGCCAGCTCGTGAT
rat mRNA     TCTGCGGAGTGCCCCTATCGCCGTCCACCTGTTTCCTCAGAAAAAAGGCCAGCTCGTGAT
113/114 NNG3
113/114 AS


             -20         -10          1          10          20          30
              |           |           |           |           |           |
Contig       CCCTGCTGCGTTCCTGGGGCCATGGCGGGTCTGGGTCCTGGCGGGGGCGACTCAGAAGGG
rat mRNA     CCCTGCTGCGTTCCTGGGGCCATGGCGGGTCTGGGTCCTGGCGGGGGCGACTCAGAAGGG
113/114 NNG3                         Start Codon
113/114 AS


              40          50          60          70          80          90
              |           |           |           |           |           |
Contig       GGACCCCGACCCCTGTTTTGCAGAAAGGGGGCGCTGAGGCAGAAGGTGGTCCACGAGGTG
rat mRNA     GGACCCCGACCCCTGTTTTGCAGAAAGGGGGCGCTGAGGCAGAAGGTGGTCCACGAGGTG
113/114 NNG3
113/114 AS


             100         110         120         130         140         150
              |           |           |           |           |           |
Contig       AAGAGCCACAAGTTCACCGCTCGTTTCTTCAAGCAGCCAACCTTCTGCAGTCACTGTACC
rat mRNA     AAGAGCCACAAGTTCACCGCTCGTTTCTTCAAGCAGCCAACCTTCTGCAGTCACTGTACC
113/114 NNG3
113/114 AS


             160         170         180         190         200         210
              |           |           |           |           |           |
Contig       GACTTCATCTGGGGCATTGGAAAGCAGGGCCTGCAATGTCAAGTCTGCAGCTTTGTGGTT
rat mRNA     GACTTCATCTGGGGCATTGGAAAGCAGGGCCTGCAATGTCAAGTCTGCAGCTTTGTGGTT
113/114 NNG3
113/114 AS


             220         230         240         250         260         270
              |           |           |           |           |           |
Contig       CACCGCCGATGCCACGAATTTGTGACCTTCGAGTGTCCAGGCGCTGGAAAGGGCCCCCAG
rat mRNA     CACCGCCGATGCCACGAATTTGTGACCTTCGAGTGTCCAGGCGCTGGAAAGGGCCCCCAG
113/114 NNG3
113/114 AS


             280         290         300         310         320         330
              |           |           |           |           |           |
Contig       ACGGACGACCCTCGCAACAAGCACAAGTTCCGTCTGCACAGCTACAGCAGTCCCACCTTC
rat mRNA     ACGGACGACCCTCGCAACAAGCACAAGTTCCGTCTGCACAGCTACAGCAGTCCCACCTTC
113/114 NNG3
113/114 AS


             340         350         360         370         380         390
              |           |           |           |           |           |
Contig       TGCGACCACTGTGGTTCCCTCCTCTACGGGCTGGTGCACCAGGGCATGAAATGTTCCTGT
rat mRNA     TGCGACCACTGTGGTTCCCTCCTCTACGGGCTGGTGCACCAGGGCATGAAATGTTCCTGT
113/114 NNG3
113/114 AS


             400         410         420         430         440         450
              |           |           |           |           |           |
Contig       TGCGAAATGAATGTGCACCGACGCTGTGTGCGCAGCGTGCCCTCCCTTTGCGGCGTGGAC
rat mRNA     TGCGAAATGAATGTGCACCGACGCTGTGTGCGCAGCGTGCCCTCCCTTTGCGGCGTGGAC
113/114 NNG3
113/114 AS


             460         470         480         490         500         510
              |           |           |           |           |           |
Contig       CATACAGAGCGCCGTGGACGTCTGCAACTGGAAATCCGGGCTCCCACATCAGATGAGATC
rat mRNA     CATACAGAGCGCCGTGGACGTCTGCAACTGGAAATCCGGGCTCCCACATCAGATGAGATC
113/114 NNG3
113/114 AS
```

FIGURE 2 (CONT)

```
          520        530        540        550        560        570
           |          |          |          |          |          |
Contig     CATATTACTGTGGGTGAGGCCCGGAACCTCATTCCTATGGACCCCAATGGCCTGTCTGAT
rat mRNA   CATATTACTGTGGGTGAGGCCCGGAACCTCATTCCTATGGACCCCAATGGCCTGTCTGAT
113/114 NNG3
113/114 AS Proof

          580        590        600        610        620        630
           |          |          |          |          |          |
Contig     CCCTATGTGAAACTGAAGCTCATCCCGGACCCTCGGAACCTGACAAAACAGAAGACAAAG
rat mRNA   CCCTATGTGAAACTGAAGCTCATCCCGGACCCTCGGAACCTGACAAAACAGAAGACAAAG
113/114 NNG3
113/114 AS

          640        650        660        670        680        690
           |          |          |          |          |          |
Contig     ACCGTGAAAGCCACACTGAATCCCGTGTGGAACGAGACCTTCGTGTTCAACCTGAAGCCG
rat mRNA   ACCGTGAAAGCCACACTGAATCCCGTGTGGAACGAGACCTTCGTGTTCAACCTGAAGCCG
113/114 NNG3
113/114 AS

          700        710        720        730        740        750
           |          |          |          |          |          |
Contig     GGGGATGTGGAGCGCCGGCTCAGTGTGGAGGTGTGGGATTGGGATAGGACATCCCGAAAT
rat mRNA   GGGGATGTGGAGCGCCGGCTCAGTGTGGAGGTGTGGGATTGGGATAGGACATCCCGAAAT
113/114 NNG3
113/114 AS

          760        770        780        790        800        810
           |          |          |          |          |          |
Contig     GACTTCATGGGTGCCATGTCCTTTGGTGTCTCAGAGCTACTCAAGGCTCCTGTGGATGGA
rat mRNA   GACTTCATGGGTGCCATGTCCTTTGGTGTCTCAGAGCTACTCAAGGCTCCTGTGGATGGA
113/114 NNG3
113/114 AS

          820        830        840        850        860        870
           |          |          |          |          |          |
Contig     TGGTACAAGTTACTGAACCAGKAGGAGGGCGAGTATTACAATGTACCGGTGGCCGATGC
rat mRNA   TGGTACAAGTTACTGAACCAGGAGGAGGGCGAGTATTACAATGTACCGGTGGCCGATGC
113/114 NNG3     ACAAGTTACTGAACCAGUAGGAGGGCGAGTATTACAATGTACCGGTGGCCGATGC
113/114 AS      ACAAGTTACTGAACCAGGAGGAGGGCGAGTATTACAATGTACCGGTGGCCGATGC

          880        890        900        910        920        930
           |          |          |          |          |          |
Contig     TGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATGA
rat mRNA   TGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATGA
113/114 NNG3 TGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATGA
113/114 AS   TGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATGA

          940        950        960        970        980        990
           |          |          |          |          |          |
Contig     GAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGA
rat mRNA   GAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGA
113/114 NNG3 GAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGA
113/114 AS   GAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGA

         1000       1010       1020       1030       1040       1050
           |          |          |          |          |          |
Contig     CTCCAAGAGATGCTTCTTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTT
rat mRNA   CTCCAAGAGATGCTTCTTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTT
113/114 NNG3 CTCCAAGAGATGCTTCTTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTT
113/114 AS   CTCCAAGAGATGCTTCTTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTT

         1060       1070       1080       1090       1100       1110
           |          |          |          |          |          |
Contig     CCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAAGGTGATGCTGGCAGARCGCAGAGGATC
rat mRNA   CCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAAGGTGATGCTGGCAGAGCGCAGAGGATC
113/114 NNG3 CCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAAGGTGATGCTGGCAGAGCGCAGAGGATC
113/114 AS   CCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAAGGTGATGCTGGCAGAACGCAGAGGATC
```

FIGURE 2 (CONT)

```
            1120    · 1130       1140       1150       1160       1170
              |         |          |          |          |          |
Contig      CGATGAACTCTATGCCATCAAGATNCTGAAAAAAGACGTCATTGTCCAGGATGATGATGT
rat mRNA    CGATGAACTCTATGCCATCAAGATACTGAAAAAAGACGTCATTGTCCAGGATGATGATGT
113/114 NNG3 CGATGAACTCTATGCCATCAAGATACTGAAAAAA
113/114 AS  CGATGAACTCTATGCCATCAAGATNCTGAAAAAA


            1180       1190       1200       1210       1220       1230
              |          |          |          |          |          |
Contig      AGACTGCACCCTTGTGGAGAAGCGTGTGCTGGCATTGGGAGGCCGAGGTCCTGGAGGCCG
rat mRNA    AGACTGCACCCTTGTGGAGAAGCGTGTGCTGGCATTGGGAGGCCGAGGTCCTGGAGGCCG
113/114 NNG3
113/114 AS


            1240       1250       1260       1270       1280       1290
              |          |          |          |          |          |
Contig      GCCACACTTTCTCACACAACTTCATTCCACCTTTCAGACTCCGGACCGCCTGTATTTTGT
rat mRNA    GCCACACTTTCTCACACAACTTCATTCCACCTTTCAGACTCCGGACCGCCTGTATTTTGT
113/114 NNG3
113/114 AS


            1300       1310       1320       1330       1340       1350
              |          |          |          |          |          |
Contig      GATGGAGTACGTCACTGGGGGCGATTTAATGTACCACATTCAGCAACTGGGCAAGTTTAA
rat mRNA    GATGGAGTACGTCACTGGGGGCGATTTAATGTACCACATTCAGCAACTGGGCAAGTTTAA
113/114 NNG3
113/114 AS


            1360       1370       1380       1390       1400       1410
              |          |          |          |          |          |
Contig      GGAGCCCCACGCAGCATTCTATGCCGCGGAAATCGCCATAGGCCTCTTCTTCCTTCACAA
rat mRNA    GGAGCCCCACGCAGCATTCTATGCCGCGGAAATCGCCATAGGCCTCTTCTTCCTTCACAA
113/114 NNG3
113/114 AS


            1420       1430       1440       1450       1460       1470
              |          |          |          |          |          |
Contig      CCAGGGCATCATCTACAGGGACCTCAAGTTGGATAATGTGATGCTGGATGCTGAAGGACA
rat mRNA    CCAGGGCATCATCTACAGGGACCTCAAGTTGGATAATGTGATGCTGGATGCTGAAGGACA
113/114 NNG3
113/114 AS


            1480       1490       1500       1510       1520       1530
              |          |          |          |          |          |
Contig      CATCAAGATCACAGACTTCGGCATGTGTAAAGAGAATGTCTTCCCTGGGTCCACAACCCG
rat mRNA    CATCAAGATCACAGACTTCGGCATGTGTAAAGAGAATGTCTTCCCTGGGTCCACAACCCG
113/114 NNG3
113/114 AS


            1540       1550       1560       1570       1580       1590
              |          |          |          |          |          |
Contig      CACCTTCTGTGGGACCCCAGACTACATAGCACCTGAGATCATTGCCTATCAGCCCTATGG
rat mRNA    CACCTTCTGTGGGACCCCAGACTACATAGCACCTGAGATCATTGCCTATCAGCCCTATGG
113/114 NNG3
113/114 AS


            1600       1610       1620       1630       1640       1650
              |          |          |          |          |          |
Contig      GAAGTCTGTCGACTGGTGGTCCTTTGGAGTCCTGCTGTATGAGATGTTGGCAGGACAGCC
rat mRNA    GAAGTCTGTCGACTGGTGGTCCTTTGGAGTCCTGCTGTATGAGATGTTGGCAGGACAGCC
113/114 NNG3
113/114 AS


            1660       1670       1680       1690       1700       1710
              |          |          |          |          |          |
Contig      ACCCTTTGATGGGGAAGATGAGGAGGAGCTGTTTCAAGCCATCATGGAACAAACTGTCAC
rat mRNA    ACCCTTTGATGGGGAAGATGAGGAGGAGCTGTTTCAAGCCATCATGGAACAAACTGTCAC
113/114 NNG3
113/114 AS


            1720       1730       1740       1750       1760       1770
              |          |          |          |          |          |
Contig      CTATCCCAAGTCACTTTCCCGGGAAGCTGTGGCCATCTGCAAGGGGTTCCTGACCAAGCA
rat mRNA    CTATCCCAAGTCACTTTCCCGGGAAGCTGTGGCCATCTGCAAGGGGTTCCTGACCAAGCA
113/114 NNG3
113/114 AS
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FIGURE 2 (CONT)

```
            1780      1790      1800      1810      1820      1830
             |         |         |         |         |         |
Contig      CCCAGGAAAGCGCCTGGGCTCAGGGCCAGATGGGGAACCCACCATCCGGGCTCATGGCTT
rat mRNA    CCCAGGAAAGCGCCTGGGCTCAGGGCCAGATGGGGAACCCACCATCCGGGCTCATGGCTT
113/114 NNG3
113/114 AS


            1840      1850      1860      1870      1880      1890
             |         |         |         |         |         |
Contig      TTTCCGTTGGATCGATTGGGAGAGGTTGGAGAGACTGGAAATTGCGCCTCCTTTTAGACC
rat mRNA    TTTCCGTTGGATCGATTGGGAGAGGTTGGAGAGACTGGAAATTGCGCCTCCTTTTAGACC
113/114 NNG3
113/114 AS


            1900      1910      1920      1930      1940      1950
             |         |         |         |         |         |
Contig      ACGTCCGTGTGGCCGCAGCGGCGAAAACTTTGACAAGTTCTTCACGCGGGCAGCGCCAGC
rat mRNA    ACGTCCGTGTGGCCGCAGCGGCGAAAACTTTGACAAGTTCTTCACGCGGGCAGCGCCAGC
113/114 NNG3
113/114 AS


            1960      1970      1980      1990      2000      2010
             |         |         |         |         |         |
Contig      CTTGACCCCGCCAGACCGCTTGGTCCTAGCCAGCATCGACCAAGCTGATTTCCAGGGCTT
rat mRNA    CTTGACCCCGCCAGACCGCTTGGTCCTAGCCAGCATCGACCAAGCTGATTTCCAGGGCTT
113/114 NNG3
113/114 AS


            2020      2030      2040      2050      2060      2070
             |         |         |         |         |         |
Contig      TACTTATGTGAACCCGGACTTCGTGCACCCAGATGCCCGCAGCCCCACAAGCCCTGTGCC
rat mRNA    TACTTATGTGAACCCGGACTTCGTGCACCCAGATGCCCGCAGCCCCACAAGCCCTGTGCC
113/114 NNG3
113/114 AS


            2080      2090      2100      2110      2120      2130
             |         |         |         |         |         |
Contig      TGTGCCCGTCATGTAATCTCATCTGCTGCCGCTAGGTGTTCCCAGTGCTCCCTCCGCCAA
rat mRNA    TGTGCCCGTCATGTAATCTCATCTGCTGCCGCTAGGTGTTCCCAGTGCTCCCTCCGCCAA
113/114 NNG3                    Stop Codon
113/114 AS


            2140      2150      2160      2170      2180      2190
             |         |         |         |         |         |
Contig      GTTGGCTGTAACTCCCATCCACCCCCATCCCCGCCTCTAGTCCGAATTTTAGGTCTCTTA
rat mRNA    GTTGGCTGTAACTCCCATCCACCCCCATCCCCGCCTCTAGTCCGAATTTTAGGTCTCTTA
113/114 NNG3
113/114 AS


            2200      2210      2220      2230      2240      2250
             |         |         |         |         |         |
Contig      AACCACCCAACCTTCTGGCCTCTTTCACGCGCCCCAAGTGGGTTCTAGACGCTGTTCCCC
rat mRNA    AACCACCCAACCTTCTGGCCTCTTTCACGCGCCCCAAGTGGGTTCTAGACGCTGTTCCCC
113/114 NNG3
113/114 AS


            2260      2270      2280      2290      2300      2310
             |         |         |         |         |         |
Contig      AGCATTGCTGGCATTTTAAACTTCAAACAGTCTCTAGAGCCTTTCTGTGTTCTAGATTCG
rat mRNA    AGCATTGCTGGCATTTTAAACTTCAAACAGTCTCTAGAGCCTTTCTGTGTTCTAGATTCG
113/114 NNG3
113/114 AS


            2320      2330      2340      2350      2360      2370
             |         |         |         |         |         |
Contig      TTGTGCTGAGCCCTGGTTTTTCCCCACCCCCAACATCTGGATGCTGTTCCAACTCTTCCC
rat mRNA    TTGTGCTGAGCCCTGGTTTTTCCCCACCCCCAACATCTGGATGCTGTTCCAACTCTTCCC
113/114 NNG3
113/114 AS


            2380      2390      2400      2410      2420      2430
             |         |         |         |         |         |
Contig      AGAAACCCCACTCCGTGTGGGGTTCTAGACTCTATCTTGGTAGTTTTATGCCTTCTCTCT
rat mRNA    AGAAACCCCACTCCGTGTGGGGTTCTAGACTCTATCTTGGTAGTTTTATGCCTTCTCTCT
113/114 NNG3
113/114 AS
```

FIGURE 2 (CONT)

```
            2440      2450      2460      2470      2480      2490
             |         |         |         |         |         |
Contig      CCCTAGACCACGTTGGGAGAAATAGTCTCATGAGATTGCCTGCTCCAGACTAAGATTCCA
rat mRNA    CCCTAGACCACGTTGGGAGAAATAGTCTCATGAGATTGCCTGCTCCAGACTAAGATTCCA
113/114 NNG3
113/114 AS

            2500      2510      2520      2530      2540      2550
             |         |         |         |         |         |
Contig      GATCAGCTCTCTGCATCCTTCAAGGCCCCTCCTACCTCCACTTCAGTTGTAGAATTAAGT
rat mRNA    GATCAGCTCTCTGCATCCTTCAAGGCCCCTCCTACCTCCACTTCAGTTGTAGAATTAAGT
113/114 NNG3
113/114 AS

            2560      2570      2580      2590      2600      2610
             |         |         |         |         |         |
Contig      GGGAGGCTGGGCTCCGTGTTCCAGGCCACCTCCCTTCCATGTTCTGGGGATTCCTGGCAT
rat mRNA    GGGAGGCTGGGCTCCGTGTTCCAGGCCACCTCCCTTCCATGTTCTGGGGATTCCTGGCAT
113/114 NNG3
113/114 AS

            2620      2630      2640      2650      2660      2670
             |         |         |         |         |         |
Contig      GCACGGAGGATTCTCTCCCCGACTTTTCTCAGTCAGCTTTTGTTCTAGATTTGTTCCAGA
rat mRNA    GCACGGAGGATTCTCTCCCCGACTTTTCTCAGTCAGCTTTTGTTCTAGATTTGTTCCAGA
113/114 NNG3
113/114 AS

            2680      2690      2700      2710      2720      2730
             |    ->    |         |         |         |         |
Contig      ACCCTTCACTGCTCACCTGCCCCGTGCATGGCTCCAGCCTTGGTCGGAATCACACACACA
rat mRNA    ACCCTTCACTGCTCACCTGCCCCGTGCATGGCTCCAGCCTTGGTCGGAATCACACACACA
113/114 NNG3
113/114 AS

            2740      2750      2760      2770      2780      2790
             |         |         |         |         |         |
Contig      CACACACACACACACACACACACACACACACACACACACACCCCTTGTCCTCCGCAGT
rat mRNA    CACACACACACACACACACACACACACACACACACACACACCCCTTGTCCTCCGCAGT
113/114 NNG3
113/114 AS

            2800      2810      2820      2830      2840      2850
             |         |    <-    |         |         |         |
Contig      GCCTGCCACTTTCTGGGACTTTCTCATCCCCCACGCCCTTCCTTTATCCTCTCCCACCCA
rat mRNA    GCCTGCCACTTTCTGGGACTTTCTCATCCCCCACGCCCTTCCTTTATCCTCTCCCACCCA
113/114 NNG3
113/114 AS

            2860      2870      2880
             |         |         |
Contig      GACACAGCTGCTGGAGAATAAATTTG
rat mRNA    GACACAGCTGCTGGAGAATAAATTTG
113/114 NNG3
113/114 AS
```

FIGURE 3

```
-141                                                              TGCTTTC
-134 TGCCCTGCGCTGCGCACCGTTAGTGCCCTGCCCCTGTCCTTCCGATCTCAGAGTCTGCGGAGTGCCC
 -67 CTATCGCCGTCCACCTGTTTCCTCAGAAAAAAGGCCAGCTCGTGATCCCTGCTGCGTTCCTGGGGCC


      Start Ala Gly Leu Gly Pro Gly Gly Gly Asp Ser Glu Gly Gly Pro Arg Pro  17
        1 ATG GCG GGT CTG GGT CCT GGC GGG GGC GAC TCA GAA GGG GGA CCC CGA CCC

      Leu Phe Cys Arg Lys Gly Ala Leu Arg Gln Lys Val Val His Glu Val Lys  34
       52 CTG TTT TGC AGA AAG GGG GCG CTG AGG CAG AAG GTG GTC CAC GAG GTG AAG

      Ser His Lys Phe Thr Ala Arg Phe Phe Lys Gln Pro Thr Phe Cys Ser His  51
      103 AGC CAC AAG TTC ACC GCT CGT TTC TTC AAG CAG CCA ACC TTC TGC AGT CAC

      Cys Thr Asp Phe Ile Trp Gly Ile Gly Lys Gln Gly Leu Gln Cys Gln Val  68
      154 TGT ACC GAC TTC ATC TGG GGC ATT GGA AAG CAG GGC CTG CAA TGT CAA GTC

      Cys Ser Phe Val Val His Arg Arg Cys His Glu Phe Val Thr Phe Glu Cys  85
      205 TGC AGC TTT GTG GTT CAC CGC CGA TGC CAC GAA TTT GTG ACC TTC GAG TGT

      Pro Gly Ala Gly Lys Gly Pro Gln Thr Asp Asp Pro Arg Asn Lys His Lys  102
      256 CCA GGC GCT GGA AAG GGC CCC CAG ACG GAC GAC CCT CGC AAC AAG CAC AAG

      Phe Arg Leu His Ser Tyr Ser Ser Pro Thr Phe Cys Asp His Cys Gly Ser  119
      307 TTC CGT CTG CAC AGC TAC AGC AGT CCC ACC TTC TGC GAC CAC TGT GGT TCC

      Leu Leu Tyr Gly Leu Val His Gln Gly Met Lys Cys Ser Cys Cys Glu Met  136
      358 CTC CTC TAC GGG CTG GTG CAC CAG GGC ATG AAA TGT TCC TGT TGC GAA ATG

      Asn Val His Arg Arg Cys Val Arg Ser Val Pro Ser Leu Cys Gly Val Asp  153
      409 AAT GTG CAC CGA CGC TGT GTG CGC AGC GTG CCC TCC CTT TGC GGC GTG GAC

      His Thr Glu Arg Arg Gly Arg Leu Gln Leu Glu Ile Arg Ala Pro Thr Ser  170
      460 CAT ACA GAG CGC CGT GGA CGT CTG CAA CTG GAA ATC CGG GCT CCC ACA TCA

      Asp Glu Ile His Ile Thr Val Gly Glu Ala Arg Asn Leu Ile Pro Met Asp  187
      511 GAT GAG ATC CAT ATT ACT GTG GGT GAG GCC CGG AAC CTC ATT CCT ATG GAC

      Pro Asn Gly Leu Ser Asp Pro Tyr Val Lys Leu Lys Leu Ile Pro Asp Pro  204
      562 CCC AAT GGC CTG TCT GAT CCC TAT GTG AAA CTG AAG CTC ATC CCG GAC CCT

      Arg Asn Leu Thr Lys Gln Lys Thr Lys Thr Val Lys Ala Thr Leu Asn Pro  221
      613 CGG AAC CTG ACA AAA CAG AAG ACA AAG ACC GTG AAA GCC ACA CTG AAT CCC

      Val Trp Asn Glu Thr Phe Val Phe Asn Leu Lys Pro Gly Asp Val Glu Arg  238
      664 GTG TGG AAC GAG ACC TTC GTG TTC AAC CTG AAG CCG GGG GAT GTG GAG CGC

      Arg Leu Ser Val Glu Val Trp Asp Trp Asp Arg Thr Ser Arg Asn Asp Phe  255
      715 CGG CTC AGT GTG GAG GTG TGG GAT TGG GAT AGG ACA TCC CGA AAT GAC TTC

      Met Gly Ala Met Ser Phe Gly Val Ser Glu Leu Leu Lys Ala Pro Val Asp  272
      766 ATG GGT GCC ATG TCC TTT GGT GTC TCA GAG CTA CTC AAG GCT CCT GTG GAT

      Gly Trp Tyr Lys Leu Leu Asn Gln Glu Glu Gly Glu Tyr Tyr Asn Val Pro  289
      817 GGA TGG TAC AAG TTA CTG AAC CAG GAG GAG GGC GAG TAT TAC AAT GTA CCG
                                           TAG
                                      Mutant NNG3 STOP

      Val Ala Asp Ala Asp Asn Cys Ser Leu Leu Gln Lys Phe Glu Ala Cys Asn  306
      868 GTG GCC GAT GCT GAC AAC TGC AGC CTC CTC CAG AAG TTT GAG GCC TGT AAT

      Tyr Pro Leu Glu Leu Tyr Glu Arg Val Arg Met Gly Pro Ser Ser Ser Pro  323
      919 TAC CCC TTG GAA TTG TAT GAG AGA GTG CGG ATG GGC CCC TCT TCC TCT CCC

      Ile Pro Ser Pro Ser Pro Ser Pro Thr Asp Ser Lys Arg Cys Phe Phe Gly  340
      970 ATT CCT TCT CCA TCC CCC AGT CCC ACG GAC TCC AAG AGA TGC TTC TTC GGT
```

FIGURE 3(CONT)

```
        Ala Ser Pro Gly Arg Leu His Ile Ser Asp Phe Ser Phe Leu Met Val Leu   357
   1021 GCC AGC CCA GGA CGC CTG CAT ATC TCT GAC TTC AGC TTC CTC ATG GTT CTA

        Gly Lys Gly Ser Phe Gly Lys Val Met Leu Ala Glu Arg Arg Gly Ser Asp   374
   1072 GGG AAA GGC AGT TTT GGG AAG GTG ATG CTG GCA GAG CGC AGA GGA TCC GAT

        Glu Leu Tyr Ala Ile Lys Ile Leu Lys Lys Asp Val Ile Val Gln Asp Asp   391
   1123 GAA CTC TAT GCC ATC AAG ATA CTG AAA AAA GAC GTC ATT GTC CAG GAT GAT

        Asp Val Asp Cys Thr Leu Val Glu Lys Arg Val Leu Ala Leu Gly Gly Arg   408
   1174 GAT GTA GAC TGC ACC CTT GTG GAG AAG CGT GTG CTG GCA TTG GGA GGC CGA

        Gly Pro Gly Gly Arg Pro His Phe Leu Thr Gln Leu His Ser Thr Phe Gln   425
   1225 GGT CCT GGA GGC CGG CCA CAC TTT CTC ACA CAA CTT CAT TCC ACC TTT CAG

        Thr Pro Asp Arg Leu Tyr Phe Val Met Glu Tyr Val Thr Gly Gly Asp Leu   442
   1276 ACT CCG GAC CGC CTG TAT TTT GTG ATG GAG TAC GTC ACT GGG GGC GAT TTA

        Met Tyr His Ile Gln Gln Leu Gly Lys Phe Lys Glu Pro His Ala Ala Phe   459
   1327 ATG TAC CAC ATT CAG CAA CTG GGC AAG TTT AAG GAG CCC CAC GCA GCA TTC

        Tyr Ala Ala Glu Ile Ala Ile Gly Leu Phe Phe Leu His Asn Gln Gly Ile   476
   1378 TAT GCC GCG GAA ATC GCC ATA GGC CTC TTC TTC CTT CAC AAC CAG GGC ATC

        Ile Tyr Arg Asp Leu Lys Leu Asp Asn Val Met Leu Asp Ala Glu Gly His   493
   1429 ATC TAC AGG GAC CTC AAG TTG GAT AAT GTG ATG CTG GAT GCT GAA GGA CAC

        Ile Lys Ile Thr Asp Phe Gly Met Cys Lys Glu Asn Val Phe Pro Gly Ser   510
   1480 ATC AAG ATC ACA GAC TTC GGC ATG TGT AAA GAG AAT GTC TTC CCT GGG TCC

        Thr Thr Arg Thr Phe Cys Gly Thr Pro Asp Tyr Ile Ala Pro Glu Ile Ile   527
   1531 ACA ACC CGC ACC TTC TGT GGG ACC CCA GAC TAC ATA GCA CCT GAG ATC ATT

        Ala Tyr Gln Pro Tyr Gly Lys Ser Val Asp Trp Trp Ser Phe Gly Val Leu   544
   1582 GCC TAT CAG CCC TAT GGG AAG TCT GTC GAC TGG TGG TCC TTT GGA GTC CTG

        Leu Tyr Glu Met Leu Ala Gly Gln Pro Pro Phe Asp Gly Glu Asp Glu Glu   561
   1633 CTG TAT GAG ATG TTG GCA GGA CAG CCA CCC TTT GAT GGG GAA GAT GAG GAG

        Glu Leu Phe Gln Ala Ile Met Glu Gln Thr Val Thr Tyr Pro Lys Ser Leu   578
   1684 GAG CTG TTT CAA GCC ATC ATG GAA CAA ACT GTC ACC TAT CCC AAG TCA CTT

        Ser Arg Glu Ala Val Ala Ile Cys Lys Gly Phe Leu Thr Lys His Pro Gly   595
   1735 TCC CGG GAA GCT GTG GCC ATC TGC AAG GGG TTC CTG ACC AAG CAC CCA GGA

        Lys Arg Leu Gly Ser Gly Pro Asp Gly Glu Pro Thr Ile Arg Ala His Gly   612
   1786 AAG CGC CTG GGC TCA GGG CCA GAT GGG GAA CCC ACC ATC CGG GCT CAT GGC

        Phe Phe Arg Trp Ile Asp Trp Glu Arg Leu Glu Arg Leu Glu Ile Ala Pro   629
   1837 TTT TTC CGT TGG ATC GAT TGG GAG AGG TTG GAG AGA CTG GAA ATT GCG CCT

        Pro Phe Arg Pro Arg Pro Cys Gly Arg Ser Gly Glu Asn Phe Asp Lys Phe   646
   1888 CCT TTT AGA CCA CGT CCG TGT GGC CGC AGC GGC GAA AAC TTT GAC AAG TTC

        Phe Thr Arg Ala Ala Pro Ala Leu Thr Pro Pro Asp Arg Leu Val Leu Ala   663
   1939 TTC ACG CGG GCA GCG CCA GCC TTG ACC CCG CCA GAC CGC TTG GTC CTA GCC

        Ser Ile Asp Gln Ala Asp Phe Gln Gly Phe Thr Tyr Val Asn Pro Asp Phe   680
   1990 AGC ATC GAC CAA GCT GAT TTC CAG GGC TTT ACT TAT GTG AAC CCG GAC TTC

        Val His Pro Asp Ala Arg Ser Pro Thr Ser Pro Val Pro Val Pro Val Met   697
   2014 GTG CAC CCA GAT GCC CGC AGC CCC ACA AGC CCT GTG CCT GTG CCC GTC ATG
```

FIGURE 3(CONT)

```
     Stop
2092 TAA TCTCATCTGCTGCCGCTAGGTGTTCCCAGTGCTCCCTCCGCCAAGTTGGCTGTAACTCCCATC
2158 CACCCCCATCCCCGCCTCTAGTCCGAATTTTAGGTCTCTTAAACCACCCAACCTTCTGGCCTCTTTC
2225 ACGCGCCCCAAGTGGGTTCTAGACGCTGTTCCCCAGCATTGCTGGCATTTTAAACTTCAAACAGTCT
2292 CTAGAGCCTTTCTGTGTTCTAGATTCGTTGTGCTGAGCCCTGGTTTTTCCCCACCCCCAACATCTGG
2359 ATGCTGTTCCAACTCTTCCCAGAAACCCCACTCCGTGTGGGGTTCTAGACTCTATCTTGGTAGTTTT
2426 ATGCCTTCTCTCTCCCTAGACCACGTTGGGAGAAATAGTCTCATGAGATTGCCTGCTCCAGACTAAG
2493 ATTCCAGATCAGCTCTCTGCATCCTTCAAGGCCCCTCCTACCTCCACTTCAGTTGTAGAATTAAGTG
2560 GGAGGCTGGGCTCCGTGTTCCAGGCCACCTCCCTTCCATGTTCTGGGGATTCCTGGCATGCACGGAG
2627 GATTCTCTCCCCGACTTTTCTCAGTCAGCTTTTGTTCTAGATTTGTTCCAGAACCCTTCACTGCTCA
2694 CCTGCCCCGTGCATGGCTCCAGCCTTGGTCGGAATCACACACACACACACACACACACACACACACA
2761 CACACACACACACACACACCACCCCTTGTCCTCCGCAGTGCCTGCCACTTTCTGGGACTTTCTCATCC
2828 CCCACGCCCTTCCTTTATCCTCTCCCACCCAGACACAGCTGCTGGAGAATAAATTTG
```

FIGURE 4

A
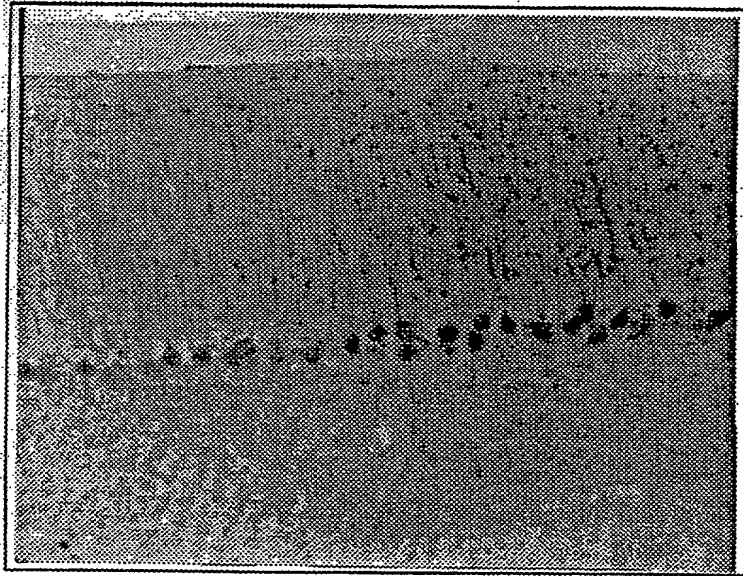
← Granule cell
layer

← Purkinje cell
layer

B
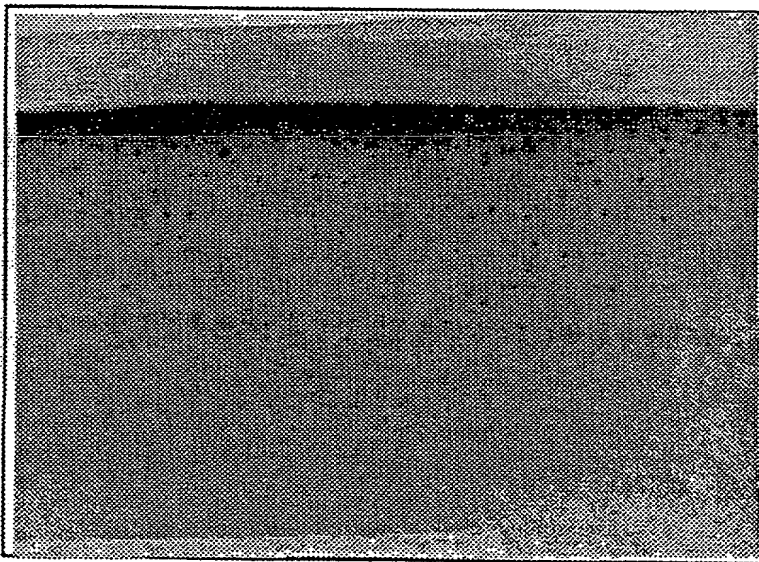
← Granule cell
layer

← Purkinje cell
layer
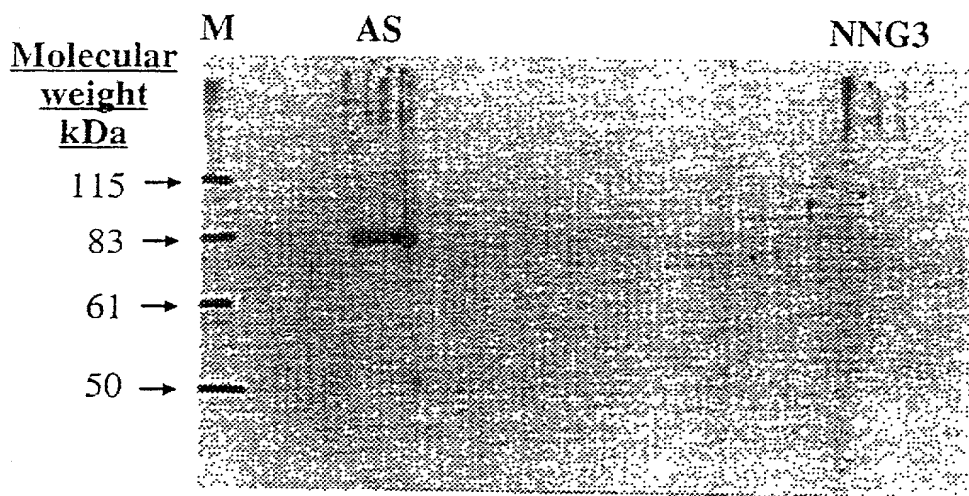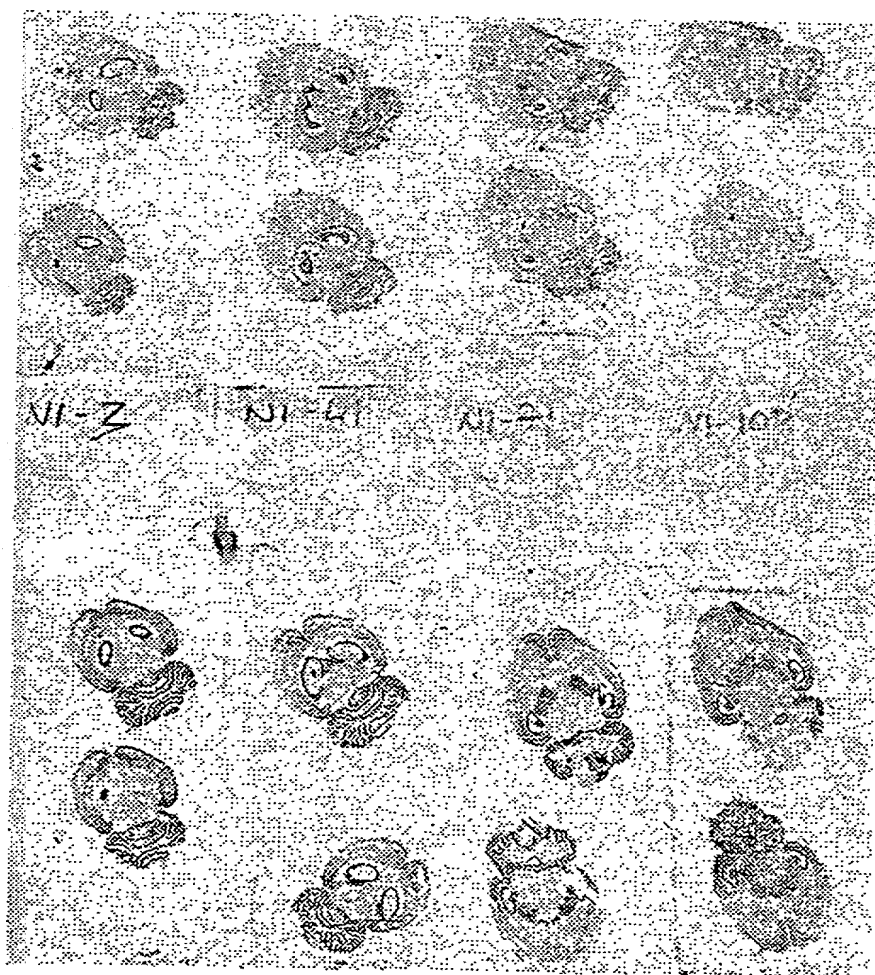
FIGURE 5

FIGURE 6

FIGURE 7

```
      -140        -130        -120        -110        -100         -90         -80         -70
       |           |           |           |           |           |           |           |
TGCTTTCTGCCCTGCGCTGCGCACCGTTAGTGCCCTGCCCCTGTCCTTCCGATCTCAGAGTCTGCGGAGTGCCCCTA
                                          ••••••••••••••••••••••••••••••••••••••
                                          TCCTTCCGATCTCAGAGTCTGCGGAGTGCCCCTA


       -60         -50         -40         -30         -20         -10          1          10
        |           |           |           |           |           |           |           |
TCGCCGTCCACCTGTTTCCTCAGAAAAAAGGCCAGCTCGTGATCCCTGCTGCGTTCCTGGGGCCATGGCGGGTCTGG
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TCGCCGTCCACCTGTTTCCTCAGAAAAAAGGCCAGCTCGTGATCCCTGCTGCGTTCCTGGGGCCATGGCGGGTCTGG
                                                                       Start codon


        20          30          40          50          60          70          80          90
         |           |           |           |           |           |           |           |
GTCCTGGCGGGGGCGACTCAGAAGGGGGACCCCGACCCCTGTTTTGCAGAAAGGGGGCGCTGAGGCAGAAGGTGGTC
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
GTCCTGGCGGGGGCGACTCAGAAGGGGGACCCCGACCCCTGTTTTGCAGAAAGGGGGCGCTGAGGCAGAAGGTGGTC


        100         110         120         130         140         150         160
         |           |           |           |           |           |           |
CACGAGGTGAAGAGCCACAAGTTCACCGCTCGTTTCTTCAAGCAGCCAACCTTCTGCAGTCACTGTACCGACTTCAT
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CACGAGGTGAAGAGCCACAAGTTCACCGCTCGTTTCTTCAAGCAGCCAACCTTCTGCAGTCACTGTACCGACTTCAT


       170         180         190         200         210         220         230         240
        |           |           |           |           |           |           |           |
CTGGGGCATTGGAAAGCAGGGCCTGCAATGTCAAGTCTGCAGCTTTGTGGTTCACCGCCGATGCCACGAATTTGTGA
••••••••••••••••••••••••••••••••••••••••  •••••••••••••••••••••••••••••••••••••
CTGGGGCATTGGAAAGCAGGGCCTGCAATGTCAGGTCTGCAGCTTTGTGGTTCACCGCCGATGCCACGAATTTGTGA


       250         260         270         280         290         300         310         320
        |           |           |           |           |           |           |           |
CCTTCGAGTGTCCAGGCGCTGGAAAGGGCCCCCAGACGGACGACCCTCGCAACAAGCACAAGTTCCGTCTGCACAGC
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CCTTCGAGTGTCCAGGCGCTGGAAAGGGCCCCCAGACGGACGACCCTCGCAACAAGCACAAGTTCCGTCTGCACAGC


       330         340         350         360         370         380         390
        |           |           |           |           |           |           |
TACAGCAGTCCCACCTTCTGCGACCACTGTGGTTCCCTCCTCTACGGGCTGGTGCACCAGGGCATGAAATGTTCCTG
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TACAGCAGTCCCACCTTCTGCGACCACTGTGGTTCCCTCCTCTACGGGCTGGTGCACCAGGGCATGAAATGTTCCTG


     400         410         420         430         440         450         460         470
      |           |           |           |           |           |           |           |
TTGCGAAATGAATGTGCACCGACGCTGTGTGCGCAGCGTGCCCTCCCTTTGCGGCGTGGACCATACAGAGCGCCGTG
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••• •••••••
TTGCGAAATGAATGTGCACCGACGCTGTGTGCGCAGCGTGCCCTCCCTTTGCGGCGTGGACCATACAGAGCGCCGTG


     480         490         500         510         520         530         540         550
      |           |           |           |           |           |           |           |
GACGTCTGCAACTGGAAATCCGGGCTCCCACATCAGATGAGATCCATATTACTGTGGGTGAGGCCCGGAACCTCATT
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
GACGTCTGCAACTGGAAATCCGGGCTCCCACATCAGATGAGATCCATATTACTGTGGGTGAGGCCCGGAACCTCATT


       560         570         580         590         600         610         620
        |           |           |           |           |           |           |
CCTATGGACCCCAATGGCCTGTCTGATCCCTATGTGAAACTGAAGCTCATCCCGGACCCTCGGAACCTGACAAAACA
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CCTATGGACCCCAATGGCCTGTCTGATCCCTATGTGAAACTGAAGCTCATCCCGGACCCTCGGAACCTGACAAAACA


  630         640         650         660         670         680         690         700
   |           |           |           |           |           |           |           |
GAAGACAAAGACCGTGAAAGCCACACTGAATCCCGTGTGGAACGAGACCTTCGTGTTCAACCTGAAGCCGGGGGATG
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
GAAGACAAAGACCGTGAAAGCCACACTGAATCCCGTGTGGAACGAGACCTTCGTGTTCAACCTGAAGCCGGGGGATG
```

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FIGURE 7 (CONT)

```
        710       720       730       740       750       760       770       780
         |         |         |         |         |         |         |         |
TGGAGCGCCGGCTCAGTGTGGAGGTGTGGGATTGGGATAGGACATCCCGAAATGACTTCATGGGTGCCATGTCCTTT
..............................................................................
TGGAGCGCCGGCTCAGTGTGGAGGTGTGGGATTGGGATAGGACATCCCGAAATGACTTCATGGGTGCCATGTCCTTT

        790       800       810       820       830       840       850       860
         |         |         |         |         |         |         |         |
GGTGTCTCAGAGCTACTCAAGGCTCCTGTGGATGGATGGTACAAGTTACTGAACCAGGAGGAGGGCGAGTATTACAA
..............................................................................
GGTGTCTCAGAGCTACTCAAGGCTCCTGTGGATGGATGGTACAAGTTACTGAACCAGGAGGAGGGCGAGTATTACAA

          870       880       890       900       910       920       930
           |         |         |         |         |         |         |
TGTACCGGTGGCCGATGCTGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATG
..............................................................................
TGTACCGGTGGCCGATGCTGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATG

     940       950       960       970       980       990      1000      1010
      |         |         |         |         |         |         |         |
AGAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGACTCCAAGAGATGCTTC
..............................................................................
AGAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGACTCCAAGAGATGCTTC

    1020      1030      1040      1050      1060      1070      1080      1090
      |         |         |         |         |         |         |         |
TTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTTCCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAA
..............................................................................
TTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTTCCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAA

         1100      1110      1120      1130      1140      1150      1160
           |         |         |         |         |         |         |
GGTGATGCTGGCAGAGCGCAGAGGATCCGATGAACTCTATGCCATCAAGATACTGAAAAAAGACGTCATTGTCCAGG
..............................................................................
GGTGATGCTGGCAGAGCGCAGAGGATCCGATGAACTCTATGCCATCAAGATACTGAAAAAAGACGTCATTGTCCAGG

  1170      1180      1190      1200      1210      1220      1230      1240
    |         |         |         |         |         |         |         |
ATGATGATGTAGACTGCACCCTTGTGGAGAAGCGTGTGCTGGCATTGGGAGGCCGAGGTCCTGGAGGCCGGCCACAC
..............................................................................
ATGATGATGTAGACTGCACCCTTGTGGAGAAGCGTGTGCTGGCATTGGGAGGCCGAGGTCCTGGAGGCCGGCCACAC

  1250      1260      1270      1280      1290      1300      1310      1320
    |         |         |         |         |         |         |         |
TTTCTCACACAACTTCATTCCACCTTTCAGACTCCGGACCGCCTGTATTTTGTGATGGAGTACGTCACTGGGGGCGA
..............................................................................
TTTCTCACACAACTTCATTCCACCTTTCAGACTCCGGACCGCCTGTATTTTGTGATGGAGTACGTCACTGGGGGCGA

    1330      1340      1350      1360      1370      1380      1390
      |         |         |         |         |         |         |
TTTAATGTACCACATTCAGCAACTGGGCAAGTTTAAGGAGCCCCACGCAGCATTCTATGCCGCGGAAATCGCCATAG
..............................................................................
TTTAATGTACCACATTCAGCAACTGGGCAAGTTTAAGGAGCCCCACGCAGCATTCTATGCCGCGGAAATCGCCATAG

 1400      1410      1420      1430      1440      1450      1460      1470
  |         |         |         |         |         |         |         |
GCCTCTTCTTCCTTCACAACCAGGGCATCATCTACAGGGACCTCAAGTTGGATAATGTGATGCTGGATGCTGAAGGA
..............................................................................
GCCTCTTCTTCCTTCACAACCAGGGCATCATCTACAGGGACCTCAAGTTGGATAATGTGATGCTGGATGCTGAAGGA

  1480      1490      1500      1510      1520      1530      1540      1550
    |         |         |         |         |         |         |         |
CACATCAAGATCACAGACTTCGGCATGTGTAAAGAGAATGTCTTCCCTGGGTCCACAACCCGCACCTTCTGTGGGAC
..............................................................................
CACATCAAGATCACAGACTTCGGCATGTGTAAAGAGAATGTCTTCCCTGGGTCCACAACCCGCACCTTCTGTGGGAC
```

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FIGURE 7 (CONT)

```
         1560      1570      1580      1590      1600      1610      1620      1630
          |         |         |         |         |         |         |         |
CCCAGACTACATAGCACCTGAGATCATTGCCTATCAGCCCTATGGGAAGTCTGTCGACTGGTGGTCCTTTGGAGTCC
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CCCAGACTACATAGCACCTGAGATCATTGCCTATCAGCCCTATGGGAAGTCTGTCGACTGGTGGTCCTTTGGAGTCC


         1640      1650      1660      1670      1680      1690      1700
          |         |         |         |         |         |         |
TGCTGTATGAGATGTTGGCAGGACAGCCACCCTTTGATGGGGAAGATGAGGAGGAGCTGTTTCAAGCCATCATGGAA
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TGCTGTATGAGATGTTGGCAGGACAGCCACCCTTTGATGGGGAAGATGAGGAGGAGCTGTTTCAAGCCATCATGGAA


         1710      1720      1730      1740      1750      1760      1770      1780
          |         |         |         |         |         |         |         |
CAAACTGTCACCTATCCCAAGTCACTTTCCCGGGAAGCTGTGGCCATCTGCAAGGGGTTCCTGACCAAGCACCCAGG
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CAAACTGTCACCTATCCCAAGTCACTTTCCCGGGAAGCTGTGGCCATCTGCAAGGGGTTCCTGACCAAGCACCCAGG


         1790      1800      1810      1820      1830      1840      1850      1860
          |         |         |         |         |         |         |         |
AAAGCGCCTGGGCTCAGGGCCAGATGGGGAACCCACCATCCGGGCTCATGGCTTTTTCCGTTGGATCGATTGGGAGA
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
AAAGCGCCTGGGCTCAGGGCCAGATGGGGAACCCACCATCCGGGCTCATGGCTTTTTCCGTTGGATCGATTGGGAGA


         1870      1880      1890      1900      1910      1920      1930
          |         |         |         |         |         |         |
GGTTGGAGAGACTGGAAATTGCGCCTCCTTTTAGACCACGTCCGTGTGGCCGCAGCGGCGAAAACTTTGACAAGTTC
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
GGTTGGAGAGACTGGAAATTGCGCCTCCTTTTAGACCACGTCCGTGTGGCCGCAGCGGCGAAAACTTTGACAAGTTC


         1940      1950      1960      1970      1980      1990      2000      2010
          |         |         |         |         |         |         |         |
TTCACGCGGGCAGCGCCAGCCTTGACCCCGCCAGACCGCTTGGTCCTAGCCAGCATCGACCAAGCTGATTTCCAGGG
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TTCACGCGGGCAGCGCCAGCCTTGACCCCGCCAGACCGCTTGGTCCTAGCCAGCATCGACCAAGCTGATTTCCAGGG


         2020      2030      2040      2050      2060      2070      2080      2090
          |         |         |         |         |         |         |         |
CTTTACTTATGTGAACCCGGACTTCGTGCACCCAGATGCCCGCAGCCCCACAAGCCCTGTGCCTGTGCCCGTCATG
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CTTTACTTATGTGAACCCGGACTTCGTGCACCCAGATGCCCGCAGCCCCACAAGCCCTGTGCCTGTGCCCGTCATG


         2100      2110      2120      2130      2140      2150      2160      2170
          |         |         |         |         |         |         |         |
TAATCTCATCTGCTGCCGCTAGGTGTTCCCAGTGCTCCCTCCGCCAAGTTGGCTGTAACTCCCATCCACCCCCATCCC
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TAATCTCATCTGCTGCCGCTAGGTGTTCCCAGTGCTCCCTCCGCCAAGTTGGCTGTAACTCCCATCCACCCCCATCCC
```
Stop Codon

```
         2180      2190      2200      2210      2220      2230      2240
          |         |         |         |         |         |         |
CGCCTCTAGTCCGAATTTTAGGTCTCTTAAACCACCCAACCTTCTGGCCTCTTTCACGCGCCCCAAGTGGGTTCTAG
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••  ••••••••••
CGCCTCTAGTCCGAATTTTAGGTCTCTTAAACCACCCAACCTTCTGGCCTCTTTCACGCGCCCCAA-TGGGTTCTAG


         2250      2260      2270      2280      2290      2300      2310      2320
          |         |         |         |         |         |         |         |
ACGCTGTTCCCCAGCATTGCTGGCATTTTAAACTTCAAACAGTCTCTAGAGCCTTTCTGTGTTCTAGATTCGTTGTG
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
ACGCTGTTCCCCAGCATTGCTGGCATTTTAAACTTCAAACAGTCTCTAGAGCCTTTCTGTGTTCTAGATTCGTTGTG
```

FIGURE 7 (CONT)

```
         2330       2340       2350       2360       2370       2380       2390       2400
          |          |          |          |          |          |          |          |
CTGAGCCCTGGTTTTTCCCCACCCCCAACATCTGGATGCTGTTCCAACTCTTCCCAGAAACCCCACTCCGTGTGGGG
..............................................................................
CTGAGCCCTGGTTTTTCCCCACCCCCAACATCTGGATGCTGTTCCAACTCTTCCCAGAAACCCCACTCCGTGTGGGG


            2410       2420       2430       2440       2450       2460       2470
             |          |          |          |          |          |          |
TTCTAGACTCTATCTTGGTAGTTTTATGCCTTCTCTCTCCCTAGACCACGTTGGGAGAAATAGTCTCATGAGATTGC
..............................................................................
TTCTAGACTCTATCTTGGTAGTTTTATGCCTTCTCTCTCCCTAGACCACGTTGGGAGAAATAGTCTCATGAGATTGC


    2480       2490       2500       2510       2520       2530       2540       2550
     |          |          |          |          |          |          |          |
CTGCTCCAGACTAAGATTCCAGATCAGCTCTCTGCATCCTTCAAGGCCCCTCCTACCTCCACTTCAGTTGTAGAA
............................................................................
CTGCTCCAGACTAAGATTCCAGATCAGCTCTCTGCATCCTTCAAGGCCCCTCCTACCTCCACTTCAGTTGTAGAA
```

FIGURE 8

```
              -190      -180      -170      -160      -150      -140
               |         |         |         |         |         |
M13977        CCTTGCGGAGCCGGCGCGCCCGGGGTGCCGCTCCCTGCCTGGCGCGCTCCGCACCTG
Human clone 28
Z15114
```

```
              -130      -120      -110      -100       -90
               |         |         |         |          |
M13977        GAGGTGCCTTGCCCCTCTCCTGCCCACCTCGGAATTTCCCTGTGGCTCCTTTGATCC
Human clone 28
Z15114                                                    CCTTTGATCC
```

```
       -80       -70       -60       -50       -40       -30
        |         |         |         |         |         |
M13977 TTCGAGTCTCCAGCTCCTCTCCCTTCCACCTGTTTCCCCCAAGAAAGGCAGGATCCT
Human clone 28
Z15114 TTCGAGTCTCCAGCTCCTCTCCCTTCCACCTGTTTCCCCCAAGAAAGGCAGGATCCT
```

```
        -20       -10        1        10        20        30
         |         |         |         |         |         |
M13977  GGTCCCTGCTACGTTTCTGGGGCCATGGCTGGTCTGGGCCCCGGCGTAGGCGATTCA
Human clone 28
Z15114  GGTCCCTGCTACGTTTCTGGGGCCATGGCTGGTCTGGGCCCCGGCGTAGGCGATTCA
```

Start codon

```
          40        50        60        70        80
           |         |         |         |         |
M13977    GAGGGGGGACCCCGGCCCCTGTTCTGCAGAAAGGGGGCTCTGAGGCAGAAGGTGGTC
Human clone 28
Z15114    GAGGGGGGACCCCGGCCCCTGTTTTGCAGAAAGGGGGCTCTGAGGCAGAAGGTGGTC
```

```
      90       100       110       120       130       140
       |         |         |         |         |         |
M13977 CACGAAGTCAAGAGCCACAAGTTCACCGCTCGCTTCTTCAAGCAGCCCACCTTCTGC
Human clone 28
Z15114 CACGAAGTCAAGAGCCACAAGTTCACCGCTCGCTTCTTCAAGCAGCCCACCTTCTGC
```

```
       150       160       170       180       190       200
        |         |         |         |         |         |
M13977 AGCCACTGCACCGACTTCATCTGGGGTATCGGAAAGCAGGGCCTGCAATGTCAAGTC
Human clone 28
Z15114 AGCCACTGCACCGACTTCATCTGGGGTATCGGAAAGCAGGGCCTGCAATGTCAAGTC
```

```
       210       220       230       240       250       260
        |         |         |         |         |         |
M13977 TGCAGCTTTGTGGTTCATCGACGATGCCACGAATTTGTGACCTTCGAGTGTCCAGGC
Human clone 28
Z15114 TGCAGCTTTGTGGTTCATCGACGATGCCACGAATTTGTGACCTTCGAGTGTCCAGGC
```

```
        270       280       290       300       310
         |         |         |         |         |
M13977  GCTGGGAAGGGCCCCCAGACGGACGACCCCCGGAACAAACACAAGTTCCGCCTGCAT
Human clone 28
Z15114  GCTGGGAAGGGCCCCCAGACGGACGACCCCCGGAACAAACACAAGTTCCGCCTGCAT
```

```
    320       330       340       350       360       370
     |         |         |         |         |         |
M13977 AGCTACAGCAGCCCCACCTTCTGCGACCACTGTGGCTCCCTCCTCTACGGGCTTGTG
Human clone 28
Z15114 AGCTACAGCAGCCCCACCTTCTGCGACCACTGTGGCTCCCTCCTCTACGGGCTTGTG
```

```
    380       390       400       410       420       430
     |         |         |         |         |         |
M13977 CACCAGGGCATGAAATGTTCTTGCTGCGAGATGAACGTGCACCGGCGCTGTGTGCG
Human clone 28
Z15114 CACCAGGGCATGAAATGCTCCTGCTGCGAGATGAACGTGCACCGGCGCTGTGTGCG
```

FIGURE 8 (CONT)

```
            440       450       460       470       480
             |         |         |         |         |
M13977          TAGCGTGCCCTCTCTGTGCGGTGTGGACCACACCGAGCGCCGCGGGCGCCTGCAGCT
Human clone 28  TAGCGTGCCCTCCCTGTGCGGTGTGGACCACACCGAGCGCCGCGGGCGCCTGCAGCT
Z15114                                                     TGCAGCT
```

```
            490       500       510       520       530       540
             |         |         |         |         |         |
M13977          GGAGATCCGGGCTCCCACAGCAGATGAGATCCACGTAACTGTTGGCGAGGCCCGTAA
Human clone 28  GGAGATCCGGGCTCCCACAGCAGATGAGATCCACGTAACTGTTGGCGAGGCCCGTAA
Z15114          GGAGATCCGGGCTCCCACAGCAGATGAGATCCACGTAACTGTTGGCGAGGCCCGTAA
```

```
            550       560       570       580       590       600
             |         |         |         |         |         |
M13977          CCTAATTCCTATGGACCCCAATGGTCTCTCTGATCCCTATGTGAAACTGAAGCTCAT
Human clone 28  CCTAATTCCTATGGACCCCAACGGTCTCTCTGATCCCTATGTGAAACTGAAGCTCAT
Z15114          CCTAATTCCTATGGACCCCAATGGTCTCTCTGATCCCTATGTGAAACTGAAGCTCAT
```

```
            610       620       630       640       650
             |         |         |         |         |
M13977          CCCAGACCCTCGGAACCTGACGAAACAGAAGACCCGAACGGTGAAAGCCACGCTAAA
Human clone 28  CCCAGACCCTCGGAACCTGACGAAACAGAAGACCCGAACGGTGAAAGCCACGCTAAA
Z15114          CCCAGACCCTCGGAACCTGACGAAACAGAAGACCCGAACGGTGAAAGCCACGCTAAA
```

```
            660       670       680       690       700       710
             |         |         |         |         |         |
M13977          CCCTGTGTGGAATGAGACCTTTGTGTTCAACCTGAAGCCAGGGGATGTGGAGCGCCG
Human clone 28  CCCTGTGTGGAATGAGACCTTTGTGTTCAACCTGAAGCCAGGGGATGTGGAGCGCCG
Z15114          CCCTGTGTGGAATGAGACCTTTGTGTTCAACCTGAAGCCAGGGGATGTGGAGCGCCG
```

```
            720       730       740       750       760       770
             |         |         |         |         |         |
M13977          GCTCAGCGTGGAGGTGTGGGACTGGGACCGGACCTCCCGCAACGACTTCATGGGGGC
Human clone 28  GCTCAGCGTGGAGGTGTGGGACTGGGACCGGACCTCCCGCAACGACTTCATGGGGGC
Z15114          GCTCAGCGTGGAGGTGTGGGACTGGGACCGGACCTCCCGCAACGACTTCATGGGGGC
```

```
            780       790       800       810       820       830
             |         |         |         |         |         |
M13977          CATGTCCTTTGGCGTCTCGGAGCTGCTCAAGGCGCCCGTGGATGGCTGGTACAAGTT
Human clone 28  CATGTCCTTTGGCGTCTCGGAGCTGCTCAAGGCGCCCGTGGATGGCTGGTACAAGTT
Z15114          CATGTCCTTTGGCGTCTCGGAGCTGCTCAAGGCGCCCGTGGATGGCTGGTACAAGTT
```

```
            840       850       860       870       880
             |         |         |         |         |
M13977          ACTGAACCAGGAGGAGGGCGAGTATTACAATGTGCCGGTGGCTGATGCTGACAACTG
Human clone 28  ACTGAACCAGGAGGAGGGCGAGTATTACAATGTGCCGGTGGCCGATGCTGACAACTG
Z15114          ACTGAACCAGGAGGAGGGCGAGTATTACAATGTGCCGGTGGCCGATGCTGACAACTG
```

```
            890       900       910       920       930       940
             |         |         |         |         |         |
M13977          CAGCCTCCTCCAGAAGTTTGAGGCTTGTAACTACCCCCTGGAATTGTATGA
Human clone 28  CAGCCTCCTCCAGAAGTTTGAGGCTTGTAACTACCCCCTGGAATTGTATGAGCGGGT
Z15114          CAGCCTCCTCCAGAAGTTTGAGGCTTGTAACTACCCCCTGGAATTGTATGAGCGGGT
```

```
            950       960       970       980       990      1000
             |         |         |         |         |         |
M13977
Human clone 28  GCGGATGGGCCCCTCTTCCTCTCCCATCCCCTCCCCTTCCCCTAGTCCCACCGACCC
Z15114          GCGGATGGGCCCCTCTTCCTCTCCCATCCCCTCCCCTTCCCCTAGTCCCACCGACCC
```

```
            1010      1020      1030      1040      1050
             |         |         |         |         |
M13977
Human clone 28  CAAGCGCTGCTTCTTCGGGGCGAGTCCAGGACGCCTGCACATCTCCGACTTCAGCTT
Z15114          CAAGCGCTGCTTCTTCGGGGCGAGTCCAGGACGCCTGCACATCTCCGACTTCAGCTT
```

FIGURE 8 (CONT)

```
              1060        1070        1080        1090        1100        1110
               |           |           |           |           |           |
M13977
Human clone 28   CCTCATGGTTCTAGGAAAAGGCAGTTTTGGGAAGGTGATGCTGGCCGAGCGCAGGGG
Z15114           CCTCATGGTTCTAGGAAAAGGCAGTTTTGGGAAGGTGATGCTGGCCGAGCGCAGGGG

              1120        1130        1140        1150        1160        1170
               |           |           |           |           |           |
M13977
Human clone 28   CTCTGATGAGCTCTACGCCATCAAGATCTTGAAAAAGGACGTGATCGTCCAGGACGA
Z15114           CTCTGATGAGCTCTACGCCATCAAGATCTTGAAAAAGGACGTGATCGTCCAGGACGA

              1180        1190        1200        1210        1220
               |           |           |           |           |
M13977
Human clone 28   CGATGTGGACTGCACGCTGGTGGAGAAACGTGTGCTGGCGCTGGGGGGCCGGGGTCC
Z15114           CGATGTGGACTGCACGCTGGTGGAGAAACGTGTGCTGGCGCTGGGGGGCCGGGGTCC

              1230        1240        1250        1260        1270        1280
               |           |           |           |           |           |
M13977
Human clone 28   TGGCGGCCGGCCCCACTTCCTCACCCAGCTCCACTCCACCTTCCAGACCCCGGACC
Z15114           TGGCGGCCGGCCCCACTTCCTCACCCAGCTCCACTCCACCTTCCAGACCCCGGACC

              1290        1300        1310        1320        1330        1340
               |           |           |           |           |           |
M13977
Human clone 28   GCCTGTATTTCGTGATGGAGTACGTCACCGGGGGAGACTTGATGTACCACATTCAAC
Z15114           GCCTGTATTTCGTGATGGAGTACGTCACCGGGGGAGACTTGATGTACCACATTCAAC

              1350        1360        1370        1380        1390        1400
               |           |           |           |           |           |
M13977
Human clone 28   AGCTGGGCAAGTTTAAGGAGCCCCATGCAGCGTTCTACGCGGCAGAAATCGCTATCG
Z15114           AGCTGGGCAAGTTTAAGGAGCCCCATGCAGCGTTCTACGCGGCAGAAATCGCTATCG

              1410        1420        1430        1440        1450
               |           |           |           |           |
M13977
Human clone 28   GCCTCTTCTTCCTTCACAATCAGGGCATCATCTACAGGGACCTGAAGCTGGACAATG
Z15114           GCCTCTTCTTCCTTCACAATCAGGGCATCATCTACAGGGACCTGAAGCTGGACAATG

              1460        1470        1480        1490        1500        1510
               |           |           |           |           |           |
M13977
Human clone 28   TGATGCTGGATGCTGAGGGACACATCAAGATCACTGACTTTGGCATGTGTAAGGAGA
Z15114           TGATGCTGGATGCTGAGGGACACATCAAGATCACTGACTTTGGCATGTGTAAGGAGA

              1520        1530        1540        1550        1560        1570
               |           |           |           |           |           |
M13977
Human clone 28   ACGTCTTCCCCGGGACGACAACCCGCACCTTCTGCGGGACCCCGGACTACATAGCCC
Z15114           ACGTCTTCCCCGGGACGACAACCCGCACCTTCTGCGGGACCCCGGACTACATAGCCC

              1580        1590        1600        1610        1620
               |           |           |           |           |
M13977
Human clone 28   CGGAGATCATTGCCTACCAGCCCTATGGGAAGTCTGTCGATTGGTGGTCCTTTGGAG
Z15114           CGGAGATCATTGCCTACCAGCCCTATGGGAAGTCTGTCGATTGGTGGTCCTTTGGAG

              1630        1640        1650        1660        1670        1680
               |           |           |           |           |           |
M13977
Human clone 28   TTCTGCTGTATGAGATGTTGGCAGGACAGCCTCCCTTCGATGGGGAGGACGAGGAGG
Z15114           TTCTGCTGTATGAGATGTTGGCAGGACAGCCTCCCTTCGATGGGGAGGACGAGGAGG
```

FIGURE 8 (CONT)

```
                  1690       1700       1710       1720       1730       1740
                   |          |          |          |          |          |
M13977
Human clone 28    AGCTGTTTCAGGCCATCATGGAACAAACTGTCACCTACCCCAAGTCGCTTTCCCGGG
Z15114            AGCTGTTTCAGGCCATCATGGAACAAACTGTCACCTACCCCAAGTCGCTTTCCCGGG


                  1750       1760       1770       1780       1790
                   |          |          |          |          |
M13977
Human clone 28    AAGCCGTGGCCATCTGCAAGGGGTTCCTGACCAAGCACCCAGGGAAGCGCCTGGGCT
Z15114            AAGCCGTGGCCATCTGCAAGGGGTTCCTGACCAAGCACCCAGGGAAGCGCCTGGGCT


                1800       1810       1820       1830       1840       1850
                 |          |          |          |          |          |
M13977
Human clone 28  CAGGGCCTGATGGGGAACCTACCATCCGTGCACATGGCTTTTTCCGCTGGATTGACT
Z15114          CAGGGCCTGATGGGGAACCTACCATCCGTGCACATGGCTTTTTCCGCTGGATTGACT


                  1860       1870       1890       1900       2000       2010
                   |          |          |          |          |          |
M13977
Human clone 28    GGGAGCGGCTGGAACGATTGGAGATCCCGCCTCCTTTCAGACCCCGCCCGTGTGGCC
Z15114            GGGAGCGGCTGGAACGATTGGAGATCCCGCCTCCTTTCAGACCCCGCCCGTGTGGCC


                  2020       2030       2040       2050       2060       2070
                   |          |          |          |          |          |
M13977
Human clone 28    GCAGCGGCGAGAACTTTGACAAGTTCTTCACGCGGGCGGCGCCAGCGCTGACCCCTC
Z15114            GCAGCGGCGAGAACTTTGACAAGTTCTTCACGCGGGCGGCGCCAGCGCTGACCCCTC


                  2080       2090       2100       2110       2120
                   |          |          |          |          |
M13977
Human clone 28    CAGACCGCCTAGTCCTGGCCAGCATCGACCAGGCCGATTTCCAGGGCTTCACCTACG
Z15114            CAGACCGCCTAGTCCTGGCCAGCATCGACCAGGCCGATTTCCAGGGCTTCACCTACG


                2130       2140       2150       2160       2170       2180
                 |          |          |          |          |          |
M13977
Human clone 28  TGAACCCCGACTTCGTGCACCCGGATGCCCGCAGCCCCACCAGCCCAGTGCCTGTGC
Z15114          TGAACCCCGACTTCGTGCACCCGGATGCCCGCAGCCCCACCAGCCCAGTGCCTGTGC


                2190       2200       2210       2220       2230       2240
                 |          |          |          |          |          |
M13977
Human clone 28  CCGTCATGTAATCTCAC
Z15114          CCGTCATGTAATCTCACCCGCCGCCACTAGGTGTCCCCAACGTCCCCTCCGCCGTGC
                **Stop codon**


                  2250       2260       2270       2280       2290
                   |          |          |          |          |
M13977
Human clone 28
Z15114            CGGCGGCAGCCCCACTTCACCCCCAACTTCACCACCCCCTGTCCCATTCTAGATCCT


                2300       2310       2320       2330       2340       2350
                 |          |          |          |          |          |
M13977
Human clone 28
Z15114          GCACCCCAGCATTCCAGCTCTGCCCCCGCGGGTTCTAGACGCCCCTCCCAAGCGTTC


                2360       2370       2380       2390       2400       2410
                 |          |          |          |          |          |
M13977
Human clone 28
Z15114          CTGGCCTTCTGAACTCCATACAGCCTCTACAGCCGTCCCGCGTTCAAGACTTGAGCG
```

FIGURE 9

```
      -140        -130        -120        -110        -100         -90         -80         -70
        |           |           |           |           |           |           |           |
TGCTTTCTGCCCTGCGCTGCGCACCGTTAGTGCCCTGCCCCTGTCCTTCCGATCTCAGAGTCTGCGGAGTGCCCCTA
                                           ...........................................
                                           CCTTCCGATCTCAGAGTCTGCGGAGTGCCCCTA


       -60         -50         -40         -30         -20         -10          1          10
        |           |           |           |           |           |           |           |
TCGCCGTCCACCTGTTTCCTCAGAAAAAAGGCCAGCTCGTGATCCCTGCTGCGTTCCTGGGGCCATGGCGGGTCTGG
...............................................................................
TCGCCGTCCACCTGTTTCCTCAGAAAAAAGGCCAGCTCGTGATCCCTGCTGCGTTCCTGGGGCCATGGCGGGTCTGG
                                                                    Start Codon


        20          30          40          50          60          70          80
         |           |           |           |           |           |           |
GTCCTGGCGGGGGCGACTCAGAAGGGGGACCCCGACCCCTGTTTTGCAGAAAGGGGGCGCTGAGGCAGAAGGTGGTC
...............................................................................
GTCCTGGCGGGGGCGACTCAGAAGGGGGACCCCGACCCCTGTTTTGCAGAAAGGGGGCGCTGAGGCAGAAGGTGGTC


        90         100         110         120         130         140        1 50         160
         |           |           |           |           |           |           |           |
CACGAGGTGAAGAGCCACAAGTTCACCGCTCGTTTCTTCAAGCAGCCAACCTTCTGCAGTCACTGTACCGACTTCAT
...............................................................................
CACGAGGTGAAGAGCCACAAGTTCACCGCTCGTTTCTTCAAGCAGCCAACCTTCTGCAGTCACTGTACCGACTTCAT


       170         180         190         200         210         220         230         240
        |           |           |           |           |           |           |           |
CTGGGGCATTGGAAAGCAGGGCCTGCAATGTCAAGTCTGCAGCTTTGTGGTTCACCGCCGATGCCACGAATTTGTGA
...............................................................................
CTGGGGCATTGGAAAGCAGGGCCTGCAATGTCAAGTCTGCAGCTTTGTGGTTCACCGCCGATGCCACGAATTTGTGA


       250         260         270         280         290         300         310         320
        |           |           |           |           |           |           |           |
CCTTCGAGTGTCCAGGCGCTGGAAAGGGCCCCCAGACGGACGACCCTCGCAACAAGCACAAGTTCCGTCTGCACAGC
...............................................................................
CCTTCGAGTGTCCAGGCGCTGGAAAGGGCCCCCAGACGGACGACCCTCGCAACAAGCACAAGTTCCGTCTGCACAGC


       330         340         350         360         370         380         390
        |           |           |           |           |           |           |
TACAGCAGTCCCACCTTCTGCGACCACTGTGGTTCCCTCCTCTACGGGCTGGTGCACCAGGGCATGAAATGTTCCTG
...............................................................................
TACAGCAGTCCCACCTTCTGCGACCACTGTGGTTCCCTCCTCTACGGGCTGGTGCACCAGGGCATGAAATGTTCCTG


       400         410         420         430         440         450         460         470
        |           |           |           |           |           |           |           |
TTGCGAAATGAATGTGCACCGACGCTGTGTGCGCAGCGTGCCCTCCCTTTGCGGCGTGGACCATACAGAGCGCCGTG
...............................................................................
TTGCGAAATGAATGTGCACCGACGCTGTGTGCGCAGCGTGCCCTCCCTTTGCGGCGTGGACCATACAGAGCGCCGTG


       480         490         500         510         520         530         540         550
        |           |           |           |           |           |           |           |
GACGTCTGCAACTGGAAATCCGGGCTCCCACATCAGATGAGATCCATATTACTGTGGGTGAGGCCCGGAACCTCATT
...............................................................................
GACGTCTGCAACTGGAAATCCGGGCTCCCACATCAGATGAGATCCATATTACTGTGGGTGAGGCCCGGAACCTCATT
```

FIGURE 9 (CONT)

```
        560       570       580       590       600       610       620
         |         |         |         |         |         |         |
CCTATGGACCCCAATGGCCTGTCTGATCCCTATGTGAAACTGAAGCTCATCCCGGACCCTCGGAACCTGACAAAACA
.............................................................................
CCTATGGACCCCAATGGCCTGTCTGATCCCTATGTGAAACTGAAGCTCATCCCGGACCCTCGGAACCTGACAAAACA


        630       640       650       660       670       680       690       700
         |         |         |         |         |         |         |         |
GAAGACAAAGACCGTGAAAGCCACACTGAATCCCGTGTGGAACGAGACCTTCGTGTTCAACCTGAAGCCGGGGGATG
.............................................................................
GAAGACAAAGACCGTGAAAGCCACACTGAATCCCGTGTGGAACGAGACCTTCGTGTTCAACCTGAAGCCGGGGGATG


        710       720       730       740       750       760       770       780
         |         |         |         |         |         |         |         |
TGGAGCGCCGGCTCAGTGTGGAGGTGTGGGATTGGGATAGGACATCCCGAAATGACTTCATGGGTGCCATGTCCTTT
.............................................................................
TGGAGCGCCGGCTCAGTGTGGAGGTGTGGGATTGGGATAGGACATCCCGAAATGACTTCATGGGTGCCATGTCCTTT


        790       800       810       820       830       840       850
         |         |         |         |         |         |         |
GGTGTCTCAGAGCTACTCAAGGCTCCTGTGGATGGATGGTACAAGTTACTGAACCAGGAGGAGGGCGAGTATTACAA
.............................................................................
GGTGTCTCAGAGCTACTCAAGGCTCCTGTGGATGGATGGTACAAGTTACTGAACCAGTAGGAGGGCGAGTATTACAA
                                                         Stop Codon

        860       870       880       890       900       910       920       930
         |         |         |         |         |         |         |         |
TGTACCGGTGGCCGATGCTGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATG
.............................................................................
TGTACCGGTGGCCGATGCTGACAACTGCAGCCTCCTCCAGAAGTTTGAGGCCTGTAATTACCCCTTGGAATTGTATG


        940       950       960       970       980       990       1000      1010
         |         |         |         |         |         |         |         |
AGAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGACTCCAAGAGATGCTTC
.............................................................................
AGAGAGTGCGGATGGGCCCCTCTTCCTCTCCCATTCCTTCTCCATCCCCCAGTCCCACGGACTCCAAGAGATGCTTC


        1020      1030      1040      1050      1060      1070      1080
         |         |         |         |         |         |         |
TTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTTCCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAA
.............................................................................
TTCGGTGCCAGCCCAGGACGCCTGCATATCTCTGACTTCAGCTTCCTCATGGTTCTAGGGAAAGGCAGTTTTGGGAA


        1090      1100      1110      1120      1130      1140      1150      1160
         |         |         |         |         |         |         |         |
GGTGATGCTGGCAGAGCGCAGAGGATCCGATGAACTCTATGCCATCAAGATACTGAAAAAAGACGTCATTGTCCAGG
.............................................................................
GGTGATGCTGGCAGAGCGCAGAGGATCCGATGAACTCTATGCCATCAAGATACTGAAAAAAGACGTCATTGTCCAGG


        1170      1180      1190      1200      1210      1220      1230      1240
         |         |         |         |         |         |         |         |
ATGATGATGTAGACTGCACCCTTGTGGAGAAGCGTGTGCTGGCATTGGGAGGCCGAGGTCCTGGAGGCCGGCCACAC
.............................................................................
ATGATGATGTAGACTGCACCCTTGTGGAGAAGCGTGTGCTGGCATTGGGAGGCCGAGGTCCTGGAGGCCGGCCACAC
```

FIGURE 9 (CONT)

```
            1250       1260       1270       1280       1290       1300       1310       1320
             |          |          |          |          |          |          |          |
TTTCTCACACAACTTCATTCCACCTTTCAGACTCCGGACCGCCTGTATTTTGTGATGGAGTACGTCACTGGGGGCGA
•••••••••••••••••••••••••••••••••• ••••••••••••••••••••••••••••••••••••••••••••
TTTCTCACACAACTTCATTCCACCTTTCAGACTCCGGGCCGCCTGTATTTTGTGATGGAGTACGTCACTGGGGGCGA


               1330       1340       1350       1360       1370       1380       1390
                |          |          |          |          |          |          |
TTTAATGTACCACATTCAGCAACTGGGCAAGTTTAAGGAGCCCCACGCAGCATTCTATGCCGCGGAAATCGCCATAG
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TTTAATGTACCACATTCAGCAACTGGGCAAGTTTAAGGAGCCCCACGCAGCATTCTATGCCGCGGAAATCGCCATAG


       1400       1410       1420       1430       1440       1450       1460       1470
        |          |          |          |          |          |          |          |
GCCTCTTCTTCCTTCACAACCAGGGCATCATCTACAGGGACCTCAAGTTGGATAATGTGATGCTGGATGCTGAAGGA
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
GCCTCTTCTTCCTTCACAACCAGGGCATCATCTACAGGGACCTCAAGTTGGATAATGTGATGCTGGATGCTGAAGGA


           1480       1490       1500       1510       1520       1530       1540       1550
            |          |          |          |          |          |          |          |
CACATCAAGATCACAGACTTCGGCATGTGTAAAGAGAATGTCTTCCCTGGGTCCACAACCCGCACCTTCTGTGGGAC
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CACATCAAGATCACAGACTTCGGCATGTGTAAAGAGAATGTCTTCCCTGGGTCCACAACCCGCACCTTCTGTGGGAC


              1560       1570       1580       1590       1600       1610       1620
               |          |          |          |          |          |          |
CCCAGACTACATAGCACCTGAGATCATTGCCTATCAGCCCTATGGGAAGTCTGTCGACTGGTGGTCCTTTGGAGTCC
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CCCAGACTACATAGCACCTGAGATCATTGCCTATCAGCCCTATGGGAAGTCTGTCGACTGGTGGTCCTTTGGAGTCC


    1630       1640       1650       1660       1670       1680       1690       1700
     |          |          |          |          |          |          |          |
TGCTGTATGAGATGTTGGCAGGACAGCCACCCTTTGATGGGGAAGATGAGGAGGAGCTGTTTCAAGCCATCATGGAA
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TGCTGTATGAGATGTTGGCAGGACAGCCACCCTTTGATGGGGAAGATGAGGAGGAGCTGTTTCAAGCCATCATGGAA


       1710       1720       1730       1740       1750       1760       1770       1780
        |          |          |          |          |          |          |          |
CAAACTGTCACCTATCCCAAGTCACTTTCCCGGGAAGCTGTGGCCATCTGCAAGGGGTTCCTGACCAAGCACCCAGG
•••••••••••••••••••• •••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CAAACTGTCACCTATCCCAAGTCACTTTCCCGGGAAGCTGTGGCCATCTGCAAGGGGTTCCTGACCAAGCACCCAGG


         1790       1800       1810       1820       1830       1840       1850
          |          |          |          |          |          |          |
AAAGCGCCTGGGCTCAGGGCCAGATGGGGAACCCACCATCCGGGCTCATGGCTTTTTCCGTTGGATCGATTGGGAGA
••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
AAAGCGCCTGGGCTCAGGGCCAGATGGGGAACCCACCATCCGGGCTCATGGCTTTTTCCGTTGGATCGATTGGGAGA


   1860       1870       1880       1890       1900       1910       1920       1930
    |          |          |          |          |          |          |          |
GGTTGGAGAGACTGGAAATTGCGCCTCCTTTTAGACCACGTCCGTGTGGCCGCAGCGGCGAAAACTTTGACAAGTTC
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
GGTTGGAGAGACTGGAAATTGCGCCTCCTTTTAGACCACGTCCGTGTGGCCGCAGCGGCGAAAACTTTGACAAGTTC
```

FIGURE 9 (CONT)

```
        1940      1950      1960      1970      1980      1990      2000      2010
          |         |         |         |         |         |         |         |
TTCACGCGGGCAGCGCCAGCCTTGACCCCGCCAGACCGCTTGGTCCTAGCCAGCATCGACCAAGCTGATTTCCAGGG
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
TTCACGCGGGCAGCGCCAGCCTTGACCCCGCCAGACCGCTTGGTCCTAGCCAGCATCGACCAAGCTGATTTCCAGGG


          2020      2030      2040      2050      2060      2070      2080
            |         |         |         |         |         |         |
CTTTACTTATGTGAACCCGGACTTCGTGCACCCAGATGCCCGCAGCCCCACAAGCCCTGTGCCTGTGCCCGTCATG
•••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
CTTTACTTATGTGAACCCGGACTTCGTGCACCCAGATGCCCGCAGCCCCACAAGCCCTGTGCCTGTGCCCGTCATG


        2090      2100      2110      2120      2130      2140      2150      2160
          |         |         |         |         |         |         |         |
TAATCTCATCTGCTGCCGCTAGGTGTTCCCAGTGCTCCCTCCGCCAAGTTGGCTGTAACTCCCATCCACCCCCATCC
••••••••••••••••••••••••••••••••••••
TAATCTCATCTGCTGCCGCTAGGTGTTCCCAGTGCTC
```

Stop Codon

```
        2170      2180      2190      2200      2210      2220      2230    2240
          |         |         |         |         |         |         |       |
CGCCTCTAGTCCGAATTTTAGGTCTCTTAAACCACCCAACCTTCTGGCCTCTTTCACGCGCCCCAAGTGGGTTCTAG


          2250      2260      2270      2280      2290      2300      2310      2320
            |         |         |         |         |         |         |         |
ACGCTGTTCCCCAGCATTGCTGGCATTTTAAACTTCAAACAGTCTCTAGAGCCTTTCTGTGTTCTAGATTCGTTGTG


          2330      2340      2350      2360      2370      2380      2390
            |         |         |         |         |         |         |
CTGAGCCCTGGTTTTTCCCCACCCCCAACATCTGGATGCTGTTCCAACTCTTCCCAGAAACCCCACTCCGTGTGGGG


        2400      2410      2420      2430      2440      2450      2460      2470
          |         |         |         |         |         |         |         |
TTCTAGACTCTATCTTGGTAGTTTTATGCCTTCTCTCTCCCTAGACCACGTTGGGAGAAATAGTCTCATGAGATTGC


          2480      2490      2500      2510      2520      2530      2540      2550
            |         |         |         |         |         |         |         |
CTGCTCCAGACTAAGATTCCAGATCAGCTCTCTGCATCCTTCAAGGCCCCTCCTACCTCCACTTCAGTTGTAGAATT


          2560      2570      2580      2590      2600      2610      2620
            |         |         |         |         |         |         |
AAGTGGGAGGCTGGGCTCCGTGTTCCAGGCCACCTCCCTTCCATGTTCTGGGGATTCCTGGCATGCACGGAGGATTC


        2630      2640      2650      2660      2670      2680      2690      2700
          |         |         |         |         |         |         |         |
TCTCCCCGACTTTTCTCAGTCAGCTTTTGTTCTAGATTTGTTCCAGAACCCTTCACTGCTCACCTGCCCCGTGCATG


          2710      2720      2730      2740      2750      2760      2770      2780
            |         |         |         |         |         |         |         |
GCTCCAGCCTTGGTCGGAATCACACACACACACACACACACACACACACACACACACACACACACACACACACCCCT


          2790      2800      2810      2820      2830      2840      2850
            |         |         |         |         |         |         |
TGTCCTCCGCAGTGCCTGCCACTTTCTGGGACTTTCTCATCCCCCACGCCCTTCCTTTATCCTCTCCCACCCAGACA


2860      2870      2880
  |         |         |
CAGCTGCTGGAGAATAAATTTG
```

FIGURE 10

```
            10        20        30        40        50        60        70
             |         |         |         |         |         |         |
MAGLGPGGGDSEGGPRPLFCRKGALRQKVVHEVKSHKFTARFFKQPTFCSHCTDFIWGIGKQGLQCQVCSFVVHRRC
...........................................................................
MAGLGPGGGDSEGGPRPLFCRKGALRQKVVHEVKSHKFTARFFKQPTFCSHCTDFIWGIGKQGLQCQVCSFVVHRRC


       80        90       100       110       120       130       140       150
        |         |         |         |         |         |         |         |
HEFVTFECPGAGKGPQTDDPRNKHKFRLHSYSSPTFCDHCGSLLYGLVHQGMKCSCCEMNVHRRCVRSVPSLCGVDH
...........................................................................
HEFVTFECPGAGKGPQTDDPRNKHKFRLHSYSSPTFCDHCGSLLYGLVHQGMKCSCCEMNVHRRCVRSVPSLCGVDH


      160       170       180       190       200       210       220       230
        |         |         |         |         |         |         |         |
TERRGRLQLEIRAPTSDEIHITVGEARNLIPMDPNGLSDPYVKLKLIPDPRNLTKQKTKTVKATLNPVWNETFVFNL
...........................................................................
TERRGRLQLEIRAPTSDEIHITVGEARNLIPMDPNGLSDPYVKLKLIPDPRNLTKQKTKTVKATLNPVWNETFVFNL
             240       250       260       270       280
              |         |         |         |         |
KPGDVERRLSVEVWDWDRTSRNDFMGAMSFGVSELLKAPVDGWYKLLNQ
.................................................
KPGDVERRLSVEVWDWDRTSRNDFMGAMSFGVSELLKAPVDGWYKLLNQEEGEYYNVPVADADNCSLLQKFEACNYP


LELYERVRMGPSSSPIPSPSPSPTDSKRCFFGASPGRLHISDFSFLMVLGKGSFGKVMLAERRGSDELYAIKILKKD
 |         |         |         |         |         |         |         |
310       320       330       340       350       360       370       380


VIVQDDDVDCTLVEKRVLALGGRGPGGRPHFLTQLHSTFQTPDRLYFVMEYVTGGDLMYHIQQLGKFKEPHAAFYAA
   |         |         |         |         |         |         |         |
  390       400       410       420       430       440       450       460


EIAIGLFFLHNQGIIYRDLKLDNVMLDAEGHIKITDFGMCKENVFPGSTTRTFCGTPDYIAPEIIAYQPYGKSVDWW
      |         |         |         |         |         |         |
     470       480       490       500       510       520       530


SFGVLLYEMLAGQPPFDGEDEEELFQAIMEQTVTYPKSLSREAVAICKGFLTKHPGKRLGSGPDGEPTIRAHGFFRW
  |         |         |         |         |         |         |         |
 540       550       560       570       580       590       600       610


IDWERLERLEIAPPFRPRPCGRSGENFDKFFTRAAPALTPPDRLVLASIDQADFQGFTYVNPDFVHPDARSPTSPVP
  |         |         |         |         |         |         |         |
 620       630       640       650       660       670       680       690


VPVM
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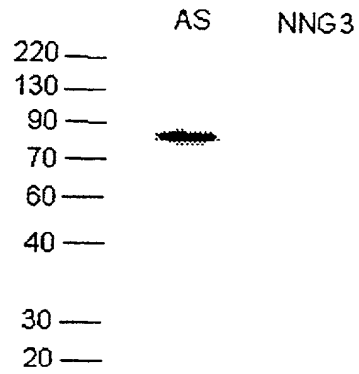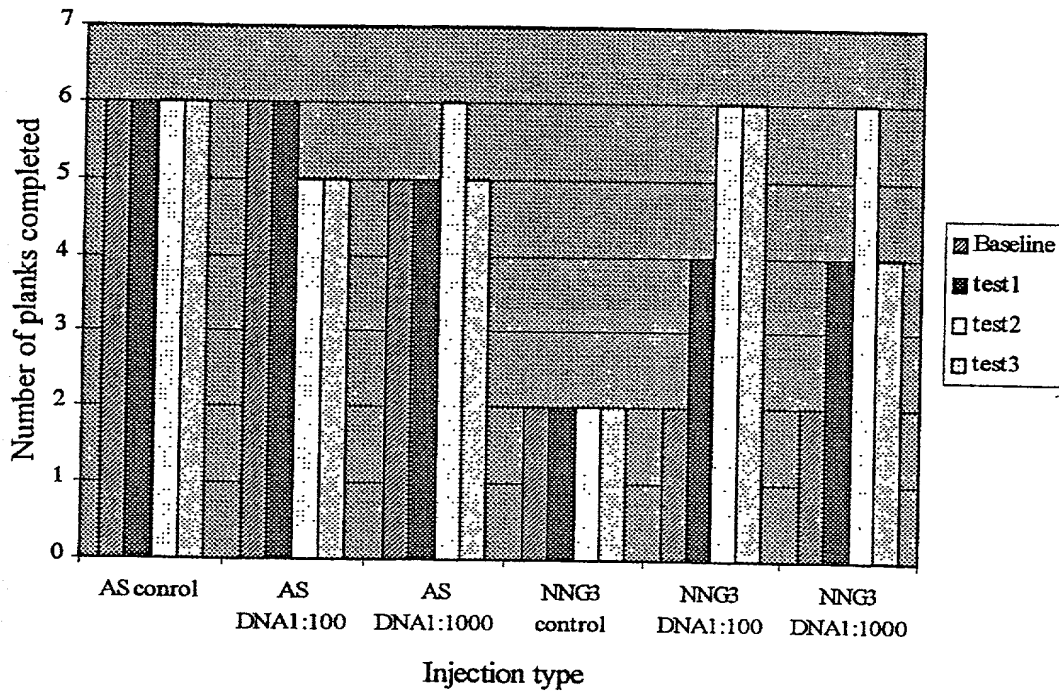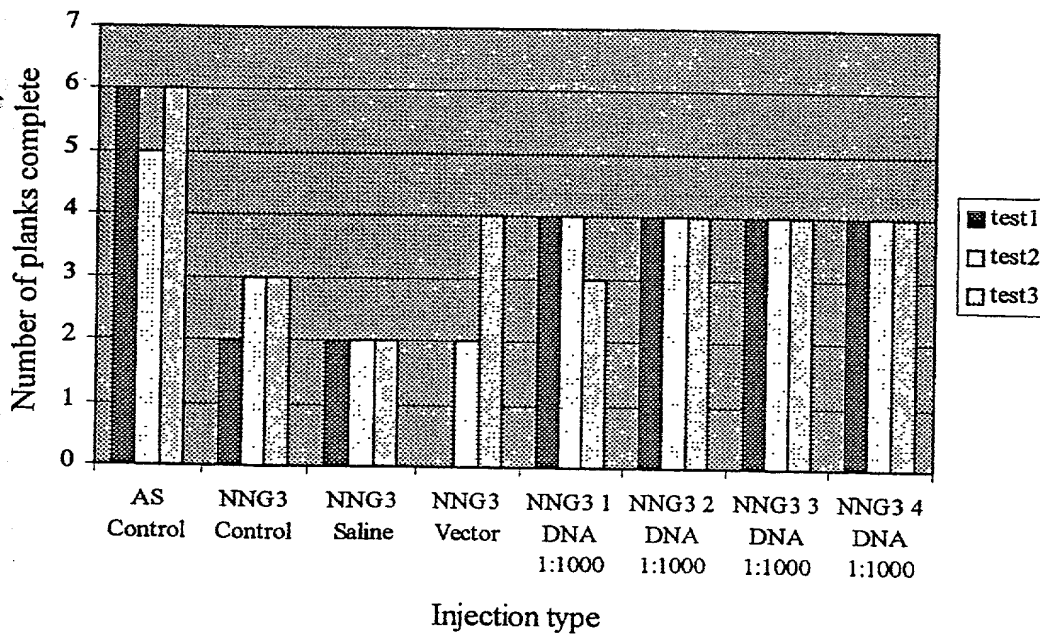
FIGURE 11

FIGURE 12
Inclined Ramp Test - experiment 1

Inclined Ramp Test - experiment 2

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

Attorney Docket No. **9013-36** _____

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled _____
"NEURODEGENERATIVE DISORDER RELATED GENE" _____ ,

the specification of which

[ ] is attached hereto

     OR

[x] was filed on _9 MARCH 200__ as United States Application No. or PCT International
Application Number ___PCT/GB00/00860___ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

9905218.5	UNITED KINGDOM	03/09/99	[X] Yes [ ] No
Number	Country	MM/DD/YYYY Filed	Priority Claimed
			[ ] Yes [ ] No
Number	Country	MM/DD/YYYY Filed	Priority Claimed
			[ ] Yes [ ] No
Number	Country	MM/DD/YYYY Filed	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)
Application Number(s)	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application (37 C.F.R. § 1.63(d)).

Appln. Serial No.	Filing Date	Status Patented/Pending/Abandoned
Appln. Serial No.	Filing Date	Status Patented/Pending/Abandoned
Appln. Serial No.	Filing Date	Status Patented/Pending/Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

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3-00

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Inventor's
Signature: _____ Date: <u>20/09/2001</u>

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Citizenship: <u>British</u>

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Full name of fourth inventor: _____

Inventor's
Signature: _____ Date: _____

Residence: _____
_____

Citizenship: _____

Post Office Address: _____
_____